AEROSOLS AND MICROBIOLOGY: CONNECTING DISCIPLINES IN THE POST-PANDEMIC ERA

4–6 June 2024

POSTER ABSTRACT BOOK

#MicroAerosols24

Mercure Bristol Grand Hotel
Broad St, Bristol BS1 2EL

MICROBIOLOGY SOCIETY

001

Investigation of novel methods to study the survival of foot-and-mouth disease virus in aerosols.

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Abstract

Foot-and-mouth disease (FMD) is one of the most important viral diseases affecting animals. The high rates of mortality in young animals, the highly contagious nature of the picornavirus, and the increase in abortions in infected animals make this a highly economical disease; annually, the cost of FMD virus (FMDV) is approximately £5.4-17.3 billion. The 2001 outbreak of FMD for example resulted in the culling of 6.5 million susceptible animals, and a cost to the UK of approximately £8 billion. The low probability-high consequence transmission of FMDV contained in aerosols (an alternative transmission route to direct inhalation) has been linked to several outbreaks, but the effect on the virus is less understood. This research will attempt to study FMDV survival within aerosols under differing environmental conditions. The CELEBS (controlled electrodynamic levitation and extraction of bioaerosols onto a substrate) instrument allows viruses to be suspended within generated aerosol particles, which are made airborne through electrodynamic levitation. These generated aerosols can be held under carefully controlled conditions within the instrument, such as a chosen temperature or humidity. By following these levitations with infectivity assays, the impact of the environmental conditions can be characterized. The results of this investigation will provide survival parameters for different contemporary strains of FMDV, additionally allowing the strains' survival to be compared. These could be used to inform outbreak policy, for example if the quarantine zones of farms containing infected animals are appropriate and account for the risk of aerosolised transmission in addition to direct transmission.

002

No pain no gain: Challenges in sampling and characterizing bioaerosols with focus on “omics” methods.

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Abstract

The role of airborne microorganisms (i.e. bioaerosols) on the environment and human health is poorly understood, due to the challenges with bioaerosol collection and characterisation. Molecular tools such as High Throughput Sequencing and “omics” approaches, have significantly advanced bioaerosol research. However, standardised (and optimised) methods to sample, detect, characterize and quantify airborne microorganisms, especially for molecular based approaches are not firmly established. Here, air filtration and liquid impingement was evaluated and optimised for bioaerosol collection for molecular analysis[1]. Specifically, we found that filtration using polycarbonate filters gave the highest DNA recovery, but the faster sampling rates with impingement using phosphate-buffered saline and centrifugation (for sample concentration), was more appropriate for fine-scale temporal/ spatial microbial community studies and which also prevented bias towards Gram-positive bacteria. Additionally we developed a method using syringe filters for rapid in-field recovery of bioaerosols from impingement samples, without compromising microbial diversity for high-throughput sequencing. These findings have fed into our review of the challenges and opportunities surrounding bioaerosol sampling and analysis. Consequently, **BioAirNet** have developed a compendium[2] of current techniques, workflows, and technologies for sampling and characterisation, to support a framework of recommendations for developing meaningful standards for better bioaerosol monitoring particularly for molecular/“omics” approaches.

References: [1.]Ferguson RMW et al (2019). Bioaerosol Biomonitoring: Sampling Optimisation for Molecular Microbial Ecology. *Mol Ecol Res*, 19:672-690. [2.]Whitby C et al (2022). Compendium of analytical methods for sampling, characterization and quantification of bioaerosols. In D. A. Bohan, & A. Dumbrell (Eds.), *Functional Microbiomes* (pp. 101-229). (Advances in Ecological Research; Vol. 67). Academic Press Inc.

003

Viral Emissions into the Air and Environment after SARS-CoV-2 Human Challenge: a Phase 1, Open Label, First-in-Human Study

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Abstract

Background

Effectively implementing strategies to curb SARS-CoV-2 transmission requires understanding who is contagious and when. SARS-CoV-2 human challenge studies provide opportunities to sample early, daily and through whole course of infection, including the incubation period.

Methods

34 young healthy adults who were unvaccinated, not previously known to have been infected with SARS-CoV-2 were recruited. Participants were inoculated intranasally with 10 $TCID_{50}$ of wild-type SARS-CoV-2. Nose and throat swabs were collected daily. Emissions were collected daily from the air (using a Coriolis sampler and directly into facemasks) and the surrounding environment (via surface and hand swabs).

Results

18 of 34 participants became infected, resulting in protracted high viral loads in the nose and throat, with mild-to-moderate symptoms. Viral RNA was detected in 63 (25%) Coriolis samples, 109 (43%) masks, 67 (27%) hand swabs, and 371 (29%) surface swabs. Viable virus was detected from 16 masks and from 13 surfaces. Viral emissions correlated more strongly with viral load in nasal than throat swabs. Two individuals emitted 86% of airborne virus. Very few emissions occurred before the first reported symptom (7%) and hardly any before the first positive lateral flow antigen test (2%).

Conclusion

After controlled experimental inoculation, the timing, extent, and routes of viral emissions was heterogeneous. A minority of participants were high airborne virus emitters, giving support to the notion of superspreading individuals or events. Our data implicates the nose as the most important source of emissions. Frequent self-testing coupled with isolation upon awareness of first symptoms could reduce onward transmissions.

004

Ambient Carbon Dioxide Concentration Correlates with SARS-CoV-2 Aerostability and Infection Risk

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Abstract

An improved understanding of the underlying physicochemical properties of respiratory aerosol that influence viral infectivity may open potential new avenues to limit the transmission of respiratory diseases such as COVID-19. Previous studies have shown that the rapid increase in the pH of such aerosols following generation is a major driver in the loss of viral infectivity. This observation has correlated with the reduction in the aerostability of successive SARS-CoV-2 variants of concern (VOC) as the virus evolved from wild type to the Delta VOC. In this study, the first instance of improved aerostability of the virus as it has evolved is reported; specifically, the BA.2 Omicron VOC is significantly more aero-stable than the Delta VOC. The consequential role of the pH sensitivity of the virus, within aerosol particles was confirmed by a dramatic increase in viral aerostability resulting from moderate (i.e. ambient) increases to atmospheric carbon dioxide concentrations, an effect more marked than that observed for changes in relative humidity. CO_2 directly affects droplet pH, and consequently viral aerostability. The effect that subtle increases in the concentration of CO_2 on the probability of COVID-19 transmission were modelled, indicating that even a moderate increase in CO_2 concentration results in a large increase in overall risk, further confirming the critical importance of ventilation in mitigating disease transmission.

005

Evaluation of Biological Aerosol Detection Systems - The Aerosol Challenge Simulator (ACS) testbed

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Abstract

The detection of bioaerosols from an unknown source in the environment is challenging. The concentration of an aerosol reduces downstream from its source dependent on the airflow vector components. This dynamic dispersion produces complex time profiles that varies with location.

The ACS has been designed to provide a controllable experimental simulation of a dispersed bioaerosol within a mixed particulate background. This capability enables high fidelity testing of aerosol instrumentation. The challenge aerosol is characterised using commercial aerosol particle sizers and UV fluorescence analysers. Physical samples can be taken using filters, cascade impactors, agar plate impactors, wetted wall cyclones and a condensation sampler.

Bioaerosols containing highly characterised Hazard Group 1 bacteria and viruses are produced from millilitre volume suspensions using a range of generation techniques. In a second identical section, a synthetic background aerosol is aerosolised from sub-gram quantities of dry dust and pollens. A mix of representative bacteria will be added in future enhancements, based on DNA sequencing from the local ambient air.

The two air streams are reduced in concentration by passing a portion of the disseminated aerosol through a HEPA filter. Repeatable temporal profiles have been demonstrated by rapid computer control of these bypass flows. The aerosols are then mixed using turbulent flows before passing into a sampling test section. Future modifications will add temperature and relative humidity control that reflects aerosol transport under a range of meteorological conditions. It is anticipated that this will represent a reproducible natural environment for the assessment of bioaerosol detection technologies.

006

Local spatiotemporal dynamics of oak pollen measured by machine learning aided optical particle counters

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Abstract

Conventional pollen monitoring techniques have significant limitations in terms of labour, cost and spatiotemporal resolution. Few studies observe airborne pollen concentrations on a local scale, even fewer do so in ecologically-rich rural areas or close to emitting sources.

We leverage machine learning techniques to estimate pollen concentrations from low-cost OPC particle size data and have trained a collection of pilot models to extract total and specific taxa pollen concentrations using data from this commercially available OPC instrument costing <€500. Here we investigate oak pollen concentrations derived from OPCs vertically distributed through a mature forest canopy and the local spatiotemporal dynamics while considering phenology and meteorology.

The time series produce a compelling picture of local oak pollen emissions, adhering to expected diurnal trends. Variation observed between the sensors due to their different positions can give insight on pollen emission through the canopy. We see that pollen concentrations are significantly higher at canopy level compared to above or below. We also evaluate the relationship between meteorological factors, phenology and observed pollen release.

This demonstrates the potential of this low-cost method to collect a wealth of information on pollen concentrations, in unique locations and at spatial resolutions that were not previously possible.

007

The airborne viability of encapsulated and acapsulate serogroup A and B *Neisseria meningitidis*

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Abstract

Neisseria meningitidis is the causative agent of meningococcal disease, characterised by severe illness and often a high case fatality rate. Despite great strides and success in vaccine development for the prevention of meningococcal disease, the transmission of *N. meningitidis* is poorly defined. 'The presence of a capsule is important for survival in the blood. We aimed to determine whether the capsule may also confer protection in the aerosol microenvironment where bacteria are subject to alkaline pH, increased salt concentration and oxidative stress. The CELEBS (Controlled Electrodynamic levitation and extraction of bioaerosol onto a substrate) instrument was used to determine bacterial viability post-aerosolization in artificial saliva. Pairs of closely related acapsulate and encapsulated strains of the same serogroup (A and B) were compared at 30, 55 and 90 relative humidity (%).

At 30 and 55RH, the capsulated serogroup A strain maintained a greater viability than its acapsulate counterpart, at 15 seconds and 5 minutes. Both strains also exhibited a dependency on relative humidity - mean viability was often significantly lower at 30RH than at 55RH and 90RH. We also compared capsulated and acapsulate strains of serogroup B *N. meningitidis* strains to determine whether a capsule with a different structure also conferred protection in the aerosol phase.

This study has begun to elucidate the airborne phase of *N. meningitidis* transmission which was previously an under-researched field. The greater airborne survival of capsulated strains suggests that the capsule may provide an advantage, not just in survival in the blood, but during transmission.

008

Assessing Aerosol Generation from Procedures and Accidents in Biological Laboratories

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Abstract

Laboratory-acquired infections (LAIs) pose a significant risk to laboratory workers and can have serious consequences. Aerosols generated during laboratory procedures are considered a significant source of LAIs, but the actual production of aerosols in many standard laboratory procedures and common accidents have not been quantified. In this study, the generation of aerosols during common laboratory procedures and accidents was assessed by conducting selected laboratory actions in a small-volume chamber with an upwelling flow. The chamber is equipped with sampling ports to simultaneously measure aerosol concentration and size distribution using a Next Generation Impactor, an Optical Particle Sizer, and an 80 mm gelatin filter. The data collected from these measurements can provide insight into the size and quantity of aerosol particles that may be generated from each procedure tested, inform the potential risk during individual procedures, and offer insights for protecting laboratory workers. The results of initial studies using a fluorescent tracer have revealed that laboratory actions can generate aerosols that could result in LAIs. Proposed future work will focus on experiments with bacterial and viral suspensions to demonstrate that infectious agents can be carried by aerosols generated in these activities.

009

Applying machine learning and statistical models to low-cost aerosol sensors for anomaly detection

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Abstract

Currently, the U.S. is investing in Biological Detection for the 21st Century that monitors for airborne releases of biological agents using anomaly detection sensors and data analytics, including machine learning algorithms to identify biological threats from background particles. Despite significant investments, background bioaerosols increase false alarm rates.

Low-cost aerosol sensors have gained popularity, including PurpleAir, which monitors particle size distribution and concentration across sensors near real-time. We hypothesize that the greater sensor concentration may enable development of advanced anomaly detection algorithms that overcome challenges with background aerosols. We have developed statistical and machine learning methods to identify anomalies within PurpleAir datasets. We have ingested a year of PurpleAir data (0.3 µM to 10 µM size ranges, temperature, and humidity) from outdoor sensors across Washington, D.C., along with meteorological conditions.

We've engineered an array of statistical and machine learning methodologies for anomaly detection within temporally aggregated data. Our approach uncovers irregularities and inconsistencies across geographic data by analyzing data patterns, time trends, relational structures, and sequential deviations. We are finalizing development of synthetic data with various meteorological conditions to simulate anomalous events by replicating the particle size distribution and concentration within a biological plume and overlaying the data on existing datasets.

We will present the different algorithm approaches that we have developed with the benefits and disadvantages of each approach in identifying the synthetic anomalous events that represent a biological attack. This includes the sensitivity, specificity, timeliness, computational requirements, and sensor density and placement requirements.

010

Promoting global collaboration to improve bioaerosol exposure assessment and understanding of associated health impacts: Outcomes from a series of workshops.

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Abstract

We are constantly inhaling air containing particles of biological origin; depending upon their size, they deposit in different parts of our airways. Despite ubiquitous exposure, the diversity and composition of bioaerosols remain inadequately characterised, and we have limited understanding of their positive or negative health impacts.

BioAirNet, one of the Clean Air Programme funded networks by the UK Research & Innovation Strategic Priorities Fund has recognised the need for the bioaerosol community to reflect on the current challenges facing bioaerosol exposure assessment and determination of the associated cellular/molecular responses driving specific health outcomes. Through professionally facilitated online workshops in September 2022, discussions considered where we need to be, where we are now and how we get there.

The workshops were attended by 32 delegates, representing different countries, sectors and disciplines. Key themes emerged: 1) Conceptual model; 2) Stakeholder mapping; 3) Knowledge transfer; 4) Writing project and 5) Conference-type event; collectively covering research, knowledge mobilisation and networking activities. An in-person follow-up meeting in November 2023 allowed for progress updates, critique, further progression and planning. Delegates also had the opportunity

to share ongoing or upcoming work, particularly projects requiring input from others, to encourage collaborative working and sharing expertise.

The use of facilitated workshops is a valuable tool for all scientific communities to collectively explore and successfully address key issues within their field.

011

Assessing the impact of aerosol degradation on microbial detection in air samples

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Abstract

Environmental air sampling is important for a range of public health and biodefense applications. Exposure to outside air may lead to irreversible changes to the surface structures and the nucleic acids of microorganisms, components that are critical for the detection of pathogens in environmental air samples. The detection of airborne microorganisms within the environment may be impacted by their physiological status. This is a consequence of the mechanical forces associated with aerosol generation and the biochemical and biophysical stresses imparted on the airborne microorganism during transport, which depends on the composition of the particle and the humidity of the surrounding environment. Research, within this project, has previously investigated the evaporative kinetics and hygroscopicity of single levitated droplets of metabolised bacterial culture media using a concentric cylindrical electrodynamic balance. The findings indicated that metabolised culture media showed differing evaporative and hygroscopic properties compared to freshly prepared (control) media droplets. Bacterial metabolism likely alters the solute composition and thus the water content of the evaporating droplet. Future studies will examine the physiological and structural changes occurring on the surface and within the nucleic acids of microorganisms associated with various aerosol generation techniques, retention under differing environmental conditions and different sampling protocols. Changes occurring to the surface and the nucleic acids will be characterised by a panel of genomic and proteomic approaches, functional assays, and molecular biology and imaging tools. This research will inform our understanding of the effectiveness of biodetection methods and support national efforts in preparedness against biological hazards.

012

Pollen sources in NAME

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Abstract

More than 10 million people in the UK suffer from hay fever. To help suffers manage their symptoms, the Met Office produces a pollen forecast. The current forecast is manually produced using expert judgement for 16 regions across the UK. Over the last few years, work has been underway to generate a model-based pollen forecast on a 0.05 degree (~5 km) grid over the UK, using the Met Office's atmospheric dispersion model, NAME. This has required the development of a representation within the model of pollen sources for the six species being modelled: alder, hazel, birch, oak, grass and nettle. This is carried out in two stages; the first uses species distribution modelling to determine environmental suitability. This is combined with estimates of the average amount of pollen produced per plant and land use maps, to generate an idealised annual map of pollen across the UK, Ireland and Northern France. The second stage determines the amount of pollen emitted at any specific time. This is derived from firstly a seasonal cycle, driven by an accumulated temperature sum. Secondly, NAME uses short-term variations in meteorology to scale the emissions, for example rainfall can reduce emissions, while higher wind speeds can increase them. This source modelling has been used as the basis for deriving pollen emissions, allowing transported and dispersed pollen air concentrations to be compared to measurements from the pollen observation network. Modelling results show a good level of skill in capturing the distribution of pollen.

013

Assessing the airborne stability of influenza A virus

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Abstract

Respiratory viruses such as Influenza A virus (IAV) pose a significant burden on UK healthcare, especially in winter months. The drivers of the seasonal increase in IAV infection rates include environmental conditions, viral genetic variation, host immunity and human behaviour. This project aims to investigate the impact of environmental conditions on IAV airborne stability, thus improving our understanding of how different environmental conditions could drive IAV seasonality.

We employ the novel Controlled Electrodynamic Levitation and Extraction of Bioaerosol onto Substrate (CELEBS) technique to assess how environmental conditions such as relative humidity (RH), temperature, and ambient CO_2 concentration impact IAV viability. This method, coupled with comparative kinetics electrodynamic balance measurements, enables inference of the microphysical processes that drive virus inactivation within aerosol droplets. We have previously reported that SARS-CoV-2 is rapidly inactivated at 40% RH due to water evaporation and subsequent spontaneous salt crystallisation. However, slow viral decay is observed at 90% RH and is thought to be due to a rapid flux of CO_2 (originally in the form of HCO_{3^-}) from the droplet, causing the droplet to become alkaline.

This project aims to compare the aerostability of IAV with that of SARS-CoV-2. Similar to SARS-CoV-2, our preliminary results indicate that IAV is rapidly inactivated at 40% RH and presents a slower inactivation rate at 90% RH. Interestingly, IAV demonstrates a quicker initial viral decay rate at 90% RH compared to SARS-CoV-2, suggesting IAV may be less aerostable than SARS-CoV-2.

014

A Systematic Review: How are Pathogens Distributed in Respiratory Emissions?

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Abstract

Pathogen laden aerosols are the mechanism of choice for a myriad of airborne transmissible diseases. Much has been done to build a picture of the physics, biology, generation and transport of these microbial ridden aerosols, in the hopes of mitigating infection risk to those susceptible. However, the initial distribution of the pathogens across the varying sizes of respiratory aerosols remains unclear, due to the demanding experimental challenges of measuring particle size segregation and those inherent when working with microorganisms. This problem is further magnified when considering the role played by evaporation in the air and the implications aerosol and droplet sizes have on the transport of the pathogens to the susceptible people. This systematic review aims to extract and collate data from the current literature in an attempt to answer the question “What is the concentration of pathogens throughout the range of aerosols sizes produced in respiratory emission”. The review is employing a systematic methodology, as laid out by PRISMA, in order to identify and examine the experimental methods capable of measuring aerosol size segregation and the quantity of pathogens/microorganisms present across the different sizes released during respiratory activities. This analysis of the literature will ultimately collate measured data to develop a better quantitative distribution of initial aerosol sizes and pathogen concentrations. The review also aims to employ bibliometric software in its analysis to help identify research gaps and connections between those working on this problem.

015

The dispersion of viral bioaerosols during dental treatment using a simulation model

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Abstract

Background: Aerosols produced by dental instruments may disperse oral microbes, risking infection. This simulation study used two bacteriophage tracers to determine the dispersion of viruses during dental procedures.

Methods: Non-enveloped MS2 ($1.3x10^{10}$ plaque-forming units [PFU]/mL) and enveloped phi6 ($3.1x10^{10}$ PFU/mL) bacteriophages were infused into a dental mannequin's mouth whilst using an air-turbine dental handpiece. Optical particle counters (OPCs) measured aerosol concentration, and 4 replicates were conducted, each with 65 surface (filter papers) and 4 air samples (BioSampler; SKC Ltd.) across a $50.3m^2$ area. Plaque assays measured infectious virus and reverse-transcription polymerase chain reaction (RT-qPCR) measured viral RNA. Non-linear regression was used to explore virus recovery at different distances.

Results: Total OPC aerosol concentration was greatest at 1m and showed ~100-fold spikes during the procedure. Infectious virus recovery reduced with increasing distance, and an average of 2,147 PFU/cm^2 was recovered from surfaces at 0.5m for MS2 and 5.3 PFU/cm^2 for phi6. Very little infectious virus was recovered from surfaces beyond 1m, although viral RNA was detectable at greater distances. Recovery of both viruses was similar in air samples at 0.5m, MS2: $1.2x10^5$ PFU/m^3; phi6: $1.0x10^5$ PFU/m^3; this reduced approximately 10-fold at 4m.

Conclusion: Infectious virus was dispersed from the mouth during dental procedures. On surfaces, the highest viral load was present within 1m, but the amount of recovered virus and transmitted distance was greater in air samples, which unmitigated, poses an infection risk. Recovery was dependent on the virus, demonstrating the importance of surrogate virus selection in simulation studies.

016

Air-seq: Rapid surveillance of the urban air microbiome

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Abstract

For several years, the authors have refined Air-seq, a whole genome nanopore sequencing approach for air microbiome surveillance. Air-seq can detect and quantify any animal, plant and microbial matter carried by the air. Importantly, it is not restricted to detecting one or two common pathogens but can identify any known organism and can flag the presence of unknown species.

Air-seq's initial focus was on agricultural pathogen surveillance, but as part of DARPA's SIGMA+ program, we deployed Air-seq to characterise the urban air microbiome around London. In two different seasons, we collected samples at 13 sites representing diverse locations. Additionally, at one site only, we collected air weekly through the whole year. Data was analysed with MARTi (Metagenomic Analysis in Real-time), our new nanopore analysis tool.

DNA yields and biodiversity varied by location and season. A wide range of taxa can be detected in urban air, including bacteria, fungi, plants and animals. At the NHMs wildlife garden, it was possible to detect DNA from many of the plant species present, but we found limitations due to reference genome availability. The same location was used for the year-round monitoring and we see clear differences across all four seasons, with plants dominating in spring and other phyla the rest of the year. Beyond species classification, we could also detect specific genes, and we generated antimicrobial resistance profiles across the year.

Whole genome sequencing of bioaerosols offers diverse potential applications including biothreat detection, surveillance of emergent threats, classifying allergens and understanding biodiversity.

017

The public health risks of bioaerosols from landspreading, composting and intensive farming- does our guidance need to change?

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Abstract

UKHSA reviews permits for regulated processes if they have the potential to impact public health from emissions, including bioaerosols or other on-site activities. The Environmental Permitting Regulations 2016 (EPR) require operators of "regulated facilities" to obtain a permit or to register some activities, which would otherwise require permits, as "exempt facilities". EPR in England is regulated by the Environment Agency or Local Authority.

UKHSA will comment on permits covering intensive poultry or pig farming applications where there are public health sensitive receptors within 100m of the site. We will also comment on composting applications and biological treatment sites.

UKHSA is undertaking a systematic literature review of intensive farming, along with composting and landspreading activities. We aim to review community exposure and health studies to find out whether relevant evidence has accumulated to change our existing guidance and position. The current position held by UKHSA is that typically, a well-managed and well-regulated intensive farm presents little risk to public health, however this has the potential to change following this review.

At this conference, we will present the findings of this review to date and outline how this will shape the guidance from UKHSA regarding public health advice on bioaerosols.

This fits into a wider body of ongoing work until March 2025, systematically reviewing bioaerosol emissions from regulated activities with a focus on wastewater treatment plants, landfill, anaerobic digestion facilities and mechanical & biological treatment, being conducted in partnership with UKHSA, the Environment Agency and the Health and Safety Executive.

018

Aerosol Science in Defence & Security at Dstl

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Abstract

Aerosol science spans many aspects of Defence & Security, spanning a range of Dstl divisions: Chemical, Biological and Radiological (CBR), Platform Systems (PLS), Cyber and Information Systems (CIS), and, Counter-Terrorism and Security (CTS). Many forms of aerosol are used, both liquid and dry powder across a range of containment environments. Aerosols and vapours are used as either the hazardous material of interest or as simulants where possible to reduce risks.

CBR Division uses aerosol science in a wide variety of projects to: understand and detect hazards; develop and evaluate protective measures and; to undertake risk and response modelling. Specific examples from Covid pandemic response include, understanding virus aerosol survival and infectivity for medical countermeasure intervention, aerosol surface deposition and touch transfer, and modelling distance particles spread from a cough including impact of mitigations (e.g. ventilation, mask wearing).

CTS Division have interests in vapours and aerosols from explosive forensics and detection perspective. Vapours may condense onto particulates becoming aerosol offering an attractive detection method. Residual trace material may transfer in the environment due to resuspension phenomena. Spray drying represents a single-step method supporting manufacturing fine particulate materials such as explosives reducing experimental variability due to material consistency.

PLS and CIS interests arise from understanding and reducing impacts of environmental particulates such as dust and volcanic ash that can impact optics and sensor technologies. Further, movement towards net zero, reduced fuel emissions and impacts of climate change means understanding effect of contrail aerosol formation and radiative effects is important to preserve mission effectiveness.

019

NATO Technical Activity: Sequencing for Environmental Aerosol Background Monitoring

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Abstract

Biological threats, including naturally occurring and modified pathogens pose a challenge to NATO operations as deployed forces may encounter endemic diseases, imported diseases, or even biological agents that are intentionally released by hostile actors. In addition, climate change is expected to accelerate the emergence or spread of zoonotic diseases, including those with pandemic potential.

Reliable and relatively fast methods for detection of harmful biological microorganisms in complex matrices are becoming more readily available through commercial, university/academic, and science and technology (S&T) defense activities. Sequencing can be used to identify any biological threat, but first, the composition of the natural background needs to be established. Biological aerosol backgrounds will vary based on location, season, time of day and meteorological conditions.

To this end, NATO has established a Research Task Group (RTG) to bring together sequencing and aerosol experts in the defense community to address challenges associated with environmental aerosol monitoring using sequencing-based approaches. The RTG seeks to broadly characterize natural backgrounds by leveraging/strategizing the NATO nation activities in this area of research. We will focus on exploring and recommending best practices for sampling and collection methods; sample preparation; sequencing technology; data analysis and interpretation of complex samples; recommendation on metrics and thresholds for improved user-confidence; and database requirements and management.

This presentation will summarize the RTG technical activity; key technical questions and issues to be addressed; individual NATO participant activities and approaches to sequencing environmental backgrounds; and future ideas, concepts, and plans for the RTG effort.

020

Automatic real-time airborne spores' identification

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Abstract

The presence of bioaerosols, including pollen and fungal spores, is a major concern for human and plant health and requires robust and precise monitoring systems. While a European norm based on the manual volumetric Hirst method exists, there's a growing interest in technologies allowing automated real-time monitoring. Over the past few years, a diverse range of automatic real-time instruments has been developed to respond to the needs of end users in terms of information about atmospheric bioaerosols. One of them, the SwisensPoleno Jupiter, is an airflow cytometer used for operational automatic bioaerosol monitoring. The instrument records holographic images and fluorescence information for single aerosol particles. This information is used to selectively identify different fungal spores using classification software based on machine learning. In this presentation we would like to show you the latest results of the automatic identification of fungal spores.

The classification capability has been explored for a series of 17 fungal species from the Belgian Collection of Fungi BCCM/IHEM, including 5 *Alternaria* species with contrasted morphological profiles. Simple classification models were developed as proof-of-principle to assess recognition capabilities. The performance obtained for classification of 8 genera by using only holography images and fluorescence measurements resulted in F1 score 0.83. Classification accuracy varied between 0.55 and 0.95 with the best performance for *Curvularia lunata* and *Alternaria* (class created as mix of 5 species). Differentiation of species was also shown to be possible for *Cladosporium*, with more difficulty for some *Alternaria* species, while the F1 score remained good (0.72).

021

Influenza A virus can be cultured from the nasal cavity from patients at relatively low RNA loads

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Abstract

The level to which an individual infected with a respiratory virus is infectious remains poorly understood. Diagnostic tests provide us Cycle threshold (Ct-) values which are semi quantitative and inversely represent the amount of genetic material present. However, unlike virus culturing, molecular detection does not represent the presence of replication competent virus. To better understand the relation between Ct values and replication competent virus in airway samples, we studied from which Ct-values influenza A virus (IAV) could be cultured. Airway samples from 100 IAV-positive patients with varying Ct-values were obtained from ErasmusMC's biobank. These samples were subsequently titrated to correlate virus culturability to Ct-values. A logistic regression analysis was performed to model the culturability based on sample Ct-values. We then proceeded to study whether the presence of IAV-neutralizing antibodies in serum reduced the relative amount of culturable virus in airway samples, as observed for SARS-CoV-2. For this a plaque-reduction neutralization test will be performed using paired airway- and serum samples collected on the same day. Overall 71/100 samples were culturable. No correlation between virus subtype (H1 or H3) and culturability was observed. Logistic regression showed a 100% chance of positive virus cultures for Ct-values below 24, which dropped to 10% at Ct 36. The relation between presence of virus-neutralizing antibodies in serum and titers is currently determined. Our study shows that Influenza A virus can be cultured from the nasal cavity from patients at a relatively low RNA load.

022

Examining mucus as a barrier to interspecies influenza virus transmission using a novel *in vitro* aerosolization inoculation method

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Abstract

Respiratory tract mucus serves as a first line defence mechanism against pathogens. Mucins are large heavily glycosylated proteins found in mucus, which can act as influenza virus decoy due to the presence of sialic acid receptors on their surface that are bound by the virus particles. To study the interaction between virus particles and mucus, we developed a virus-mucus inhibition assay and a virus-mucus penetration model for human and ferret mucus. The latter is based on aerosolization of influenza viruses above a confluent cell layer overlayed with mucus in an in-house developed aerosol exposure chamber. This set-up allows us to mimic a more natural aerosol-like way of inoculation of cells, compared to the conventional liquid inoculation. Ferret and human airway epithelial cells in an air-liquid interface were established to produce mucus. To assess differences in virus-mucus interaction between several virus subtypes isolated from different hosts, human, avian, and swine H1N1 and H3N2 influenza viruses, were studied. The virus-mucus inhibition assay showed different inhibition patterns for the human and swine viruses, while no inhibition for the avian influenza virus was observed regardless of the mucus type. These observations were confirmed in the virus-mucus penetration assay. Furthermore, differences in inhibition patterns were observed between ferret and human mucus in both assays, showing that virus inhibition by mucus can be species-specific. Overall, this study demonstrates the use of our novel *in vitro* inoculation method based on virus aerosolization and shows that mucus can serve as a barrier to interspecies influenza virus transmission.

023

Designing and validating a novel aerosol chamber to assess respiratory virus viability kinetics in the air

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Abstract

Respiratory viruses can be transmitted via (in-)direct contact and through air. However, the relative contribution of each route to population-wide spread is unknown. Therefore, a comprehensive understanding of how long viruses remain infectious in the air and what environmental factors influence their viability is needed. Such knowledge is crucial for implementing accurate mitigation strategies during future outbreaks and pandemics. Unfortunately, there is no standardised *in vitro* system for analysing respiratory virus viability in the air.

Here, we present a novel, in-house-developed aerosol chamber for artificially aerosolising viruses in physiologically relevant substances like mucus derived from air-liquid-interface cultures from human airway epithelial cells. Following aerosolization, virus-laden droplets are maintained suspended in the chamber for predetermined periods before being sampled using various air samplers.

Characterisation of the droplet size distribution in the aerosol chamber using phase doppler anemometry showed a consistent mean droplet diameter of around 3 µm, although droplet size ranged from 0.1 to 20 µm. This suggests a realistic heterogeneity as observed in exhaled breath. Through infectivity assays on collected aerosols, we distinguished different degrees of viability kinetics and decay among four common respiratory viruses (influenza virus, human metapneumovirus, respiratory syncytial virus, and parainfluenza virus), which may indicate different transmission risks. These observations are currently being confirmed in a large systemic analysis.

Our findings provide insight into the viability and associated transmission risk of prototype viruses from respiratory virus families with pandemic potential setting a benchmark for comparison with newly emerging viruses that may cause future outbreaks and pandemics.

024

Development of the ferret as a model for inhalation exposure to the encephalitic alphaviruses.

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Abstract

We sought to evaluate the ferret as a model for aerosol infection with Venezuelan equine encephalitis virus (VEEV) and eastern equine encephalitis (EEEV). Ferrets were implanted with radiotelemetry devices to monitor temperature and activity, then infected with VEEV or EEEV by small particle aerosol. For comparison, a second set of ferrets was inoculated subcutaneously with VEEV or EEEV to mimic mosquito transmission. By aerosol, VEEV caused a biphasic fever in the ferrets with a pattern very similar to what has been observed in cynomolgus macaques. Ferrets exposed to aerosolized VEEV succumbed within 5-8 days at all doses tested. High levels of virus were found throughout the CNS. Subcutaneous VEEV also caused a biphasic fever but only 2 of 6 succumbed at 10,000 pfu. For aerosolized EEEV, a fever was noted within 1 day of infection and ferrets succumbed within 5-7 days. IVIS imaging showed evidence of viral replication in the CNS. At necropsy, virus was found throughout the CNS but at the highest levels in the cerebellum and spinal cord. Viral RNA but not infectious virus was found in the CNS of surviving ferrets. After subcutaneous inoculation with EEEV, in contrast, very little fever was seen and none of the ferrets succumbed.

The disease course in the ferret after aerosol infection is similar to what we and others have previously observed and reported in the cynomolgus macaque, and what has been reported in humans. The ferret appears to be a relevant model of human disease, suitable for pivotal efficacy studies.

025

“Insights into Mycobacterium abscessus: Exploring ColonyMorphotypes, Hydrophobicity, and Transmission Dynamics”

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Abstract

Mycobacterium abscessus (Mab), identified in 1953 from a knee abscess (type strain Mab ATCC 19977), is an opportunistic pathogen causing severe infections, notably in cystic fibrosis patients with pre-existing lung conditions. While initially thought to originate from environmental sources, recent evidence suggests patient-to-patient transmission. Mab exhibits two colony morphotypes: Smooth (S) and Rough (R), with S strains producing high levels of glycopeptidolipid (GPL) and R strains showing reduced or absent GPL production. The transition from S to R involves mutations abolishing GPL expression, potentially impacting cellular hydrophobicity and aerosol transmission.

Comparative analysis of type and clinical S and R variants revealed distinct growth patterns in broth and biofilm assays, with R strains consistently displaying higher hydrophobicity and lacking GPL production. Luciferase-expressing strains were constructed to facilitate investigation of desiccation and UV resistance. No significant differences between S and R were observed in desiccation experiments, while the S strain showed greater resistance to UV exposure. Interestingly, when the two strains were mixed, they showed similar hydrophobicities, possibly indicating redistribution of GPLs in the suspension.

Genome and transcriptome analyses identified numerous polymorphisms associated with R transition, indicating a broader genomic impact than previously recognized. Overall, findings provided limited support for R strains being adapted to airborne transmission, highlighting a wider range of genomic targets involved in the S to R transition. This work extends our knowledge of Mab factors linked to pathogenicity and transmission dynamics and emphasizes the complex interplay between colony morphotypes, hydrophobicity, and genetic factors.

026

Measuring viral decay in single particle study systems: Overcoming the limitations of low viral titers

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Abstract

When using single particle levitation techniques such as CELEBS (Controlled electrodynamic levitation and extraction of bioaerosol onto a substrate) to study airborne viral decay, there are some unique challenges one must consider;

1. Propagating virus concentrations required to generate statistically relevant data

2. Maintaining reproducible aerosol compositions that reflect respiratory secretions

The CELEBS study system uses aerosol phase droplets of highly reproducible size, generated using a droplet on demand dispenser. However, in droplets of such small volumes (~70 picoliters) there are limitations associated when measuring the viral concentration, and consequently inferring viral decay. For example, to consistently generate droplets containing at least one infectious particle per droplet we require an initial viral concentration of $2x10^7$ infectious units.ml^{-1}.

Here we report a ten-fold increase of infectious unit per droplet in coronavirus measurements through adjustments to multiplicity of infection and viral incubation rather than changing the composition of aerosolized sample i.e. through centrifugal concentration. This avoids unquantifiable adjustments to droplet evaporation dynamics and consequently airborne virus stability measurements. Furthermore, we report how the propagation of influenza in serum pathogen free (SPF) embryonated eggs achieves an increased infectious unit per droplet compared with tissue culture methods, although allantoic fluid not a traditional surrogate of respiratory fluid.

We outline how an increased virus per droplet can mitigate inaccuracy when reporting viral decay measurements in high time resolution single particle levitation systems. With this in mind, viral stock generation, quantification and aerosolisation must be consistent to avoid uncertainty and improve reproducibility between viral aerostability measurements.

027

Towards a household quantitative microbial risk assessment model for SARS-CoV-2

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Abstract

Background
The accuracy of quantitative models of SARS-CoV-2 aerosol and fomite transmission could be increased by incorporating longitudinal data of viral environmental contamination, which have recently become available.

Methods
We developed a quantitative aerosol and fomite transmission model to evaluate the impact of public health interventions and parameter uncertainty on the estimated transmission risk, utilising daily measurements of viral RNA contamination of air and surfaces in a SARS-CoV-2 human infection challenge study. Model parameters included the virus decay rate, surface-to-skin viral transfer efficiency, behavioural patterns, and the dose-dependent risk of infection, which was estimated using previous primate dose-response studies of SARS-CoV-2.

Results
The modelled secondary attack rates through the airborne and fomite routes were consistent with that observed in household studies of SARS-CoV-2 transmission. Index-case mask-wearing was found to be the most effective intervention in reducing the risk of fomite transmission, followed by more frequent surface cleaning and hand washing. Air filtration was effective in reducing airborne virus transmission risk. More precise empirical measurements of the frequency of touching fomites, viral transfer efficiency, the fraction of the fomite touched, and the fraction of virus on hands located on fingertips may reduce the uncertainty of the estimated transmission risk.

Conclusion
Our empirically supported, quantitative model of SARS-CoV-2 aerosol and fomite transmission may be used to evaluate public health interventions and inform quantitative microbial risk assessment (QMRA) models. Incorporating plans for measuring identified key parameters and longitudinal viral contamination of air and surfaces during respiratory virus outbreaks could strengthen pandemic preparedness programs.

028

Refinement of aerobiology techniques for the pre-clinical evaluation of interventions against respiratory pathogens

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Abstract

Respiratory infectious diseases remain a growing global health crisis to humans. New vaccines and therapeutics are needed to reduce deaths. The UKHSA prepares for improved public health outcomes through vaccination and responds to save lives by reducing the impact of antimicrobial resistance via new therapies. UKHSA have established advanced models of respiratory pathogen infections to pre-clinically evaluate interventions against diseases of public concern. Such diseases include Tuberculosis, Plague, Melioidosis, Q fever, Anthrax and Monkeypox.

Aerobiology strategies have been refined to establish delivered doses (including ultra-low doses) of bacterial pathogens to individually challenged animals and separately challenged groups of animals within high containment laboratory facilities. The study of the aerobiological characteristics of micro-organisms is a critical step in the development of robust aerosol infection animal models. Pathogens are initially characterised to understand their ability to withstand aerosolisation and subsequent loss in viability within aerosols over time. A modified Henderson apparatus is used in conjunction with an AeroMP controller/Aero3G software and aerosol particles containing pathogens are delivered using a nose-only system specifically adapted for each species.

UKHSA collaborate globally with vaccine and therapeutic developers to provide pre-clinical safety and efficacy data for progression of candidates towards clinical trials in humans. Establishing disease in the appropriate model by the aerosol route is key to mimicking the human disease state. Refined protocols for pathogen characterisation and aerosol infection significantly increases accuracy and reproducibility of infection leading to significant reductions in the size of studies required to produce the data to save human lives.

029

Aerosol Science: Multidisciplinary Capability & Expertise Across UKHSA

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Abstract

The United Kingdom Health Security Agency (UKHSA) exists to protect our communities from infectious diseases and the impact of chemical, radiological and other environmental health hazards. As part of our role, we provide advice on and conduct research into the health effects of a range of inhaled aerosols, including infectious agents and air pollution components, including bioaerosols.

At Chilton we use inhalation suites and molecular laboratories to investigate health impacts on a wide range of aerosols, including consumer products, e-cigarette vape, microplastics, nanoparticles, combustion particulates, other particulate and gaseous air pollutants and bioaerosols. This involves comprehensive characterisation of human relevant aerosol exposures and the use of complex cell and rodent models to determine their associated effects on the airways, gut or brain.

At Porton, aerobiology techniques are used to characterise respiratory and opportunistic pathogens in aerosol particles, and to develop and establish controlled aerosol infections at high containment for the pre-clinical evaluation of interventions (vaccines and therapeutics) against all respiratory diseases that threaten public health. Commercial services are provided to manufacturers in healthcare and biopharma, including testing of medical devices, air filtration/disinfection products.

UKHSA collaborate globally with government, academia and industry on a wide range of aerosol related projects across multiple disciplines. The application of aerosol science expertise is essential to UKHSA, and we recruit specialist aerosol scientists to support these activities. An aim of UKHSA is to ensure the UK drives innovation in health protection and life sciences in collaboration with government, academia, and industry.

030

Aerodynamic Particle Size Influences the Infectious Dose of Aerosolized SARS-CoV-2 in a Hamster Model of Inhalational COVID-19

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Abstract

The aim of the present study was to examine the role of aerosol particle size on the dose-infectivity relationship for SARS-CoV-2 in a golden Syrian hamster model of inhalational COVID-19. Animals were exposed to aerosols containing SARS-CoV-2 with mass median aerodynamic diameters of either 1 or 5 µm, and disease presentation was monitored for 28 days post-exposure. Positron emission tomography-computed tomography was also utilized to quantify deposition of aerosols containing ^{18}F-fluorodeoxyglucose to examine differences in deposition patterns as a function of particle size. In SARS-CoV-2-exposed animals, an increase in aerosol particle size increased the median doses required to induce seroconversion and viral shedding by approximately 30-fold. Infected hamsters also demonstrated decreased activity and weight gain, and an increase in respiratory rate, although these effects were similar between animals exposed to the different particle sizes. These results suggest that aerodynamic particle size is an important factor that needs to be considered when modeling respiratory transmission of disease. Analysis of regional deposition of aerosols within the respiratory tract of hamsters is currently ongoing but will be discussed along with potential future avenues of research to better understand the host mechanisms responsible for the particle size dependent differences in the infectivity of bioaerosols.

This work was funded under Contract No. HSHQDC-15-C-00064, awarded by the DHS S&T to NBACC, a DHS federal laboratory operated by BNBI. Views and conclusions contained herein are those of the authors and should not be interpreted to represent policies, expressed or implied, of the DHS or S&T.

031

Seasonal variations during long-term measurements of fungal and bacterial bioaerosols in a coniferous forest in the South of Sweden

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Abstract

Bioaerosols are essential for microbial and gene dispersal and have been proposed as important agents for atmospheric processes. However, the abundance and size distributions of atmospheric biological particles are largely unknown. In this study we used a laser-induced fluorescence instrument to measure fluorescent biological aerosol particle (FBAP) concentrations for 18 months (October 2020–April 2022) at a rural, forested site in Sweden. We investigated relationships between number concentration of FBAP (NFBAP) and meteorological parameters. In addition, filter sampling was performed in parallel for analysis by microscopy.

NFBAP was highest in summer and lowest in winter, exhibiting a ~5-fold difference between these seasons.

The median NFBAP was 0.0050, 0.0025, 0.0027, and 0.0126 cm^{-3} in fall, winter, spring, and summer, respectively, and constituted ~0.1–0.5% of the total supermicron particle number concentration. NFBAP was dominated by the smallest measured size fraction (1–3 µm), suggesting that we mainly measured single bacterial cells, fungal spores, and bacterial agglomerates. There was a significant correlation between the bioaerosol concentration measured on filters and the N_{FBAP} (ρ=62, P<0.0001), however, the bioaeosol concentration on filters was about 10 times higher than N_{FBAP}.

Our results indicate that NFBAP was highest during warm and dry conditions when wind speeds were high, suggesting that a major part of the FBAP in spring and summer was due to mechanical aerosol generation and release mechanisms. This is one of the longest time series of atmospheric FBAPs, which are greatly needed for estimates of bioaerosol background concentrations in comparable regions.

032

Advancing workplace safety: A South African perspective on research into hazardous biological agents

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Abstract

Bioaerosols can contain biological particles such as bacteria, fungi, viruses, secondary metabolites of fungi and other particles of plant and animal origin. These agents are often respirable and may lead to occupational diseases where workers are intentionally or accidentally exposed to these biological agents. The COVID-19 pandemic has heightened awareness of disease outbreaks originating from exposure to hazardous biological agents (HBAs) as part of bioaerosols. In South Africa, the Regulations Hazardous Biological Agents Regulations were promulgated by the Minister of Employment and Labour on 16 March 2022 in terms of section 43 of the Occupational Health and Safety Act (OHSA), 1993 and state that employers are to control the exposure to HBAs in the workplace via various reasonably practicable measures. In addition to bioaerosol awareness and the updated HBA Regulations, molecular and genomic analyses of biological agents have also progressed significantly in recent years. In light of these advances, the objective of the current study was to perform a literature review on the number of research studies done in South African workplaces on hazardous biological agents before and after the COVID-19 pandemic. Factors such as increased awareness and technological advances are considered. This study emphasizes the importance of ongoing research on hazardous biological agents in the workplace. It outlines future directions for research, including anticipated trends and areas warranting further exploration in the face of evolving biological hazards.

033

Dielectrophoretic collection of airborne particles

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Abstract

Bioaerosols impact human health, animal well-being, and crop yield through airborne transmission. Electrostatic aerosol collection techniques use corona discharge which can affect bioaerosol viability and lead to sample loss. Dielectrophoretic collection onto a liquid film may offer an alternative approach to address these challenges.

To investigate dielectrophoretic actuation, ITO slides were connected to four plates, each at different forced potential (negative, positive, grounded and floating) inside an $8m^3$ aerosol chamber. Potential differences of between 2.5kV-10kV were applied to the plates during 15-minute collection runs in presence of aerosolised fluorescent polystyrene latex beads. An optical particle counter was used to monitor aerosol concentration. The average particle count was quantified using fluorescence microscopy and normalised against the chamber concentration.

Samples connected to the positive or negative bias displayed a significant increase in the collection performance compared to the grounded and floating plates. Up to 96% of collected particles in a run were detected on samples connected to positive and negative voltage. The normalised collected particle count increased with the voltage, e.g. +10kV collected 7749 particles (normalised) whilst +2.5kV collected 1744 particles (normalised). On average, the positively biased plates collected approximately 20% more particles than the negatively biased. Grounded and floating plates collected similar numbers of particles with an average of 384 particles (normalised).

This research demonstrates that dielectrophoretic collection can potentially be used to collect bioaerosols without impacting their viability. Further investigation is required to demonstrate the efficiency of dielectrophoretic collection of biological aerosols into liquid films and validate the sample viability.

034

Evaluation of an electrostatic bioaerosol collector with digital microfluidics sample recovery.

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Abstract

Early identification of airborne biological threats is paramount in protecting health and informing strategic responses. This research compares the performance of a prototype aerosol collection system, comprising of a personal electrostatic precipitator aerosol sampler, combined with an electrowetting on dielectric sample recovery system (ESP-EWOD), against two standard bioaerosol collectors.

The ESP-EWOD, a SKC BioSampler®, and a 37 mm filter cassette containing a 0.8 µm polycarbonate filter were tested in a GenaMini bioaerosol chamber. Three bacteria (Bacillus atrophaeus spores, Pantoea agglomerans, and Escherichia coli) were aerosolised into the chamber. The samples were collected and eluted for culture, endotoxin assay and quantitative polymerase chain reaction (qPCR) using specific targets.

Semi-quantitative comparison showed that ESP-EWOD has comparable collection and elution efficiency as the BioSampler® and filter cassette for E. coli and P. agglomerans, but reduced elution efficiency with B. atrophaeus spores. However, crucially ESP-EWOD sample concentrations were 1000x higher for E. coli and P. agglomerans and 33x higher than those collected with the SKC BioSampler® for B. atrophaeus spores.

This first in a series of planned trials showed that the ESP-EWOD is a promising technology for bioaerosol detector system integration. Further work will focus on optimising the ESP-EWOD system to improve collection and elution efficiency and to explore the effects of Tween surfactants on the system performance. Future planned tests include field trials in easily accessible locations with complex environmental backgrounds such as animal barns or wastewater treatment plants, to ascertain new application areas for the ESP-EWOD aerosol collector technology.

035

Droplet size differentiation in facemask sampling

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Abstract

Face mask sampling (FMS) is a novel method using masks containing polyvinyl alcohol (PVA) inserts to capture exhaled materials. By investigating individuals with respiratory infections, FMS studies in clinical and non-clinical settings have shown their utility for community-level sampling and clear capacity to asses source infectiousness in both tuberculosis (PMID: 36350995) and COVID-19 (PMID: 35843566). However, the question of the size of droplets captured and contributing to positive signals remains open.

To investigate the distribution of droplets within masks, sections of the PVA inserts were extracted separately to determine the distribution evenness of exhaled microbial signals. An arbitrary threshold was chosen for even (possible aerosol) versus uneven (possible large droplets) between the PVA insert segments. Results indicate significant differences between individuals and analyses relating these data to household transmissions are in progress.

Droplet size was addressed further using a formed plastic (Mayku) mask designed by Caroline Marshall and Waseem Hiwar (University of Leeds). This mask presents two chambers separated by a baffle enabling, in principle, collection of large droplets in the first chamber and aerosol in the second. The masks have been used to sample two individuals with COVID-19. Much lower signals E gene were detected in the second chamber and the implications of these findings will be discussed.

FMS provides opportunities to measure source strength in different particle sizes from individuals in non-clinical settings where transmission can be assessed.

036

Optimising the aerosolization and detection of microorganisms for industry applications.

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Abstract

Bioaerosols containing biological components such as bacteria, viruses and fungi are generated through a multitude of activities and there are numerous health risks associated with them. Under most circumstances, bioaerosols pose a low threat to healthy individuals, however, there are multiple environments and industries where the risks of exposure and potential resultant ill-health effects are considerably higher. For example, waste treatment facilities and healthcare settings. It is therefore of interest to measure bioaerosols within these higher-risk environments.

This project, in partnership between InBio and Cardiff Metropolitan university, is focused on investigating the ability of the ambient air sampling device developed by InBio (Apollo), to detect airborne microorganisms for application within industry settings. Results already obtained for Apollo have shown increased sensitivity for aeroallergen detection compared to other commonly used methods of detection for both indoor and food allergens. Results have also demonstrated the ability of the device to detect airborne endotoxin via Endozyme ® II rFC assay.

Current work being undertaken involves optimising the aerosolization of industry relevant bacteria, viruses and fungi from liquid cultures within the ozone chamber at Cardiff Metropolitan University, followed by sampling the air with both Apollo and an impactor, for comparison, to measure their detection capabilities. Samples are then enumerated via culture techniques. Apollo is an easy-to-use air sampling device and testing its ability to detect microorganisms is of potential interest to multiple industries where there is increased chance of, and risk associated with bioaerosols.

037

Particle size effects on the short term aerosol decay of Escherichia coli MRE 162

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Abstract

Knowledge of how particle size influences the survival of airborne pathogens can inform risk mitigation strategies for disease prevention. *Escherichia coli* MRE162 was used as a surrogate to characterise a new experimental system to understand early phase decay 30 seconds from generation and the associated effects on post-generation evaporation kinetics.

A bespoke 1504mm system was built and characterised with sodium fluorescein, a non-biological tracer. Four 275 mm sections and a 390 mm Sonotek section were used to enable use of three differing particle size nozzles: The Lee, the 120kHz Sonotek and the 60kHz Sonotek. All samples were collected with 2 different filters and 3 types of impingers.

Characterisation of the experimental system will be described: aerosol particle size distribution (APSD) profiles are characterised via particle mass median aerodynamic diameter (MMAD) and Geometric standard deviation (GSD).

Using the bespoke system, no loss of viability attributed to particle size was noted at 30 second early evaporation stages following experimental aerosolisation of *E. coli* MRE 162. This demonstrates the utility of this experimental approach to characterise the effects of particle size on early phase microbial decay and its applications in the future to assess early phase decay of pathogens to inform risk mitigation.

038

The use of Gaseous Ozone for Bioaerosol Disinfection.

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Abstract

Gaseous ozone has come to light over recent years as a potential decontamination agent for bioaerosols in settings where the management of airborne microorganisms is crucial such as in the healthcare and food industry. Ozone, an inorganic molecule has strong oxidative properties, acting as an antimicrobial agent through the induction of reactive oxygen species oxidative stress. With its rapid action, auto decomposition and oxidative capabilities, the use of ozone has gained significant interest.

The Ozone Research Group at Cardiff Metropolitan University investigates the efficacy of ozone through various approaches. One approach involves utilising the bioaerosol chamber within the university to assess the effectiveness of gaseous ozone against bioaerosols. Situated in a biosafety level 2 laboratory, the chamber is equipped with an ozone generator, monitor and humidity controls, enabling the examination of ozone efficacy under diverse humidity controls, which impacts the effectiveness of ozone. Industry-relevant microorganisms are aerosolised within the chamber and gaseous ozone is introduced at a specific concentration controlled using a 2B technologies ozone monitor. Air samples are collected over 4 hours using an impactor to quantify microorganism levels, allowing for efficacy determination through comparison with control samples.

Primarily data indicates that gaseous ozone, maintained at a concentration of 5 ppm under 90% relative humidity, achieves a 4-log reduction in Staphylococcus aureus levels following a 20-minute exposure. These results suggest ozone holds significant promise as a method of bioaerosol disinfection.

039

“An Optimized Active Sampling Procedure for Aerobiological DNA Studies”

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Abstract

Although, real-time genomic studies can provide a sensitive and rapid method for monitoring the microbial composition of bioaerosols, the amount of biomass in the air suspension being too low makes it extremely difficult to monitor the changes over time in these communities. The low abundance of deoxyribose nucleic acid (DNA) and proteins in the atmosphere, which is of the order of the contamination produced by operators and instruments, poses a challenge for the sampling process and the analyte extraction. In this study, we designed an optimized, portable, closed bioaerosol sampler based on membrane filters using commercial off-the-shelf components, demonstrating its end-to-end operation. This sampler can operate autonomously outdoors for a prolonged time, capturing ambient bioaerosols and avoiding user contamination. We first performed a comparative analysis in a controlled environment to select the optimal active membrane filter based on its ability to capture and extract DNA. We designed a bioaerosol chamber for this purpose and tested three commercial DNA extraction kits and four filters. The bioaerosol sampler was tested outdoors in a representative environment and run for 24 h at 150 L/min. Our methodology suggests that a 0.22 µm polyether sulfone (PES) membrane filter can recover up to 4 ng of DNA in this period, sufficient for genomic applications. Using MinION nanopore sequencer (Oxford Nanopore Technologies), we sequenced one of the samples without amplification and found airborne microorganisms mainly *Escherichia coli*, human contamination, *Pseudomonas* etc. This system, along with the robust extraction protocol, can be automated for continuous environmental monitoring.

040

FLUID DYNAMICS INSIGHTS ON AEROSOL GENERATION IN THE RESPIRATORY TRACT

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Abstract

Determining the presence of pathogens in the air expelled during respiration offers a non-invasive avenue to diagnose respiratory diseases. As these pathogens are trapped in the aerosol generated during respiration, identifying the size and source of aerosol particles can provide information about the infected regions of the respiratory tract. Physiological studies have identified several sources of these aerosols: vocal chord vibrations, saliva break-up due to vocalisation and, mucus film break-up due to contraction and expansion of bronchioles and alveoli. However, there is limited clarity on size distribution and quantity of aerosol particles generated due to these mechanisms.

The formation of aerosol particles involves complex interactions of air flow, structural dynamics of the respiratory tract and liquid properties of mucus and saliva films, such as, surface tension, film thickness and viscoelasticity. In this work, we shed light on the fundamental mechanisms of aerosol generation by examining the underlying fluid dynamic interactions. Using simple scaling principles, we analyse how aerosol size distributions can vary based on break-up of liquid films due to vibrations (~100µm) and air flow over thin liquid films (~10-100µm) and filaments (~0.1-100µm) as a direct analogy to the physical mechanisms occurring in the respiratory tract. We highlight the influence of viscoelastic and surface properties of the mucus lining throughout the respiratory tract, and the airflow dynamics through these varied structures. The physical principles highlighted in this work provide an understanding of the physiological principles of aerosol generation and can lead to potential pathways for precise treatment of respiratory disease.

041

Pulmonary aerosol viral loads in acute COVID-19 are 85.1x higher than salivary loads

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Abstract

The relative risk of upper vs lower respiratory tract aerosols in SARS-CoV-2 transmission remains unclear. We used 3D-printed prototypes of PBM-HALE™ to remove inertially impacted aerosols 3 cm from the mouth, before condensing only the terminal 48mL of each exhalation.

Acute COVID-19 patients (days 0-5 from symptom onset, n=30; 10 healthy controls) were recruited under informed consent at a Brazilian favela 1ry healthcare centre (UFMG IRB approval 54358021.1.0000.5149). Saliva, nasopharyngeal swabs, condensates of tidal breathing (30 min), and singing happy birthday (15 min) were collected. Viral load was determined by CDC N1/N2/RP multiplex RT-PCR normalised against 18S rRNA (Applied Biosystems) on RNA extracts (PureLink™). Salivary alpha-amylase activity was assessed enzymatically (Salimetrix), and condensate immunoprofiling by Luminex.

COVID-19 patients offered 30/30 tidal and 28/30 singing samples; 4 sample sets suffered RNA extraction contamination (24 complete datasets). Salivary amylase was detectable only in saliva (40/40) and inertially impacted droplets (20/68). Condensate yield (0.113±0.05 mL/min) was independent of exhalation mode (p=0.205).

Singing increased condensate [18S rRNA] 90.6x to Ct 27.8±1.90 (no RP detected) against saliva Ct 19.6±1.36 and swab Ct 21.8±2.63. COVID-19 patient singing condensates had normalised SARS-CoV-2 RNA levels (24/24 positive) 85.1x higher vs saliva (25/26 positive; Spearman correlation p=0.0013), and 1.47x higher vs swabs (26/26 positive; Spearman correlation p=0.084) with no healthy participant false positives, and featured significant cytokine changes (IL-4=68.6x, Holm-Šidák Mann-Whitney $-\log_{10}(p)=5.56$); IL-12(p40)=31.7x, $-\log_{10}(p)=1.63$; IL-8=0.131x, $-\log_{10}(p)=1.56$; TNF-a=4.29x, $-\log_{10}(p)=1.44$).

Distal airway aerosols evidence lung immune responses and have 1.93 $\log_{10}$ higher viral loads than saliva.

042

Aerosol transmission of *Mycobacterium abscessus* and its Cell Surface Hydrophobicity

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Abstract

Mycobacterium abscessus is an opportunistic pathogen responsible for cutaneous and pulmonary infections in immunocompromised patients and patients with chronic lung disease, such as cystic fibrosis. This rapid-growing mycobacterium is found in water, soil, and dust. The main and exact environmental reservoir of this organism remains unknown. *M. abscessus* exhibits two distinct morphotypes, smooth (S) and rough (R). The irreversible transition is known to happen from a S variant with cell surface-associated glycopeptidolipids (GPL) to a R variant lacking GPL. We hypothesise that *M. abscessus* cell surface hydrophobicity (CSH) plays a key role in the aerosol transmission.

Building on the work of Dr Al-Mkhadhree (see poster), a detailed picture of *M. abscessus* CSH values for both morphotypes has been established using hexadecane partitioning in a Microbial Adhesion To Hydrocarbon (MATH) assay. This measures the reduction of culture density in the aqueous phase after mixing with hexadecane. Bacilli with high CSH tend to move into hydrocarbon layer, while cells with low CSH remain in the aqueous solution. We have begun to test our proposal that more hydrophobic cells are more easily aerosolised and transmitted using an Omron nebuliser and a Biosampler collection system. The complications encountered, results obtained and challenges faced in testing this hypothesis will be discussed.

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