The removal of airborne SARS-CoV-2 and other microbial bioaerosols by air filtration on COVID-19 surge units

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Key words

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Summary

Airborne SARS-CoV-2 was detected in a COVID-19 ward before activation of portable HEPA-air filtration, but not during the week of filter operation; SARS-CoV-2 was again detected when the filter was off. Airborne SARS-CoV-2 was infrequently detected in a COVID-19 ICU. Filtration significantly reduced other microbial bioaerosols in both settings.

Introduction

Airborne dissemination is likely an important transmission route for SARS-CoV-2[1], with SARS-CoV-2 RNA detected in air samples from COVID-19 wards[1,2]. Despite the use of personal protective equipment (PPE), there are multiple reports of patient-to-healthcare worker transmission of SARS-CoV-2[3], potentially through the inhalation of viral particles[4] . There is a need to improve the safety for healthcare workers and patients by decreasing airborne transmission of SARS-CoV-2[4]. Portable air filtration systems, that combine high efficiency particulate filtration and ultraviolet (UV) light sterilisation, may be a scalable solution for removing respirable SARS-CoV-2[5]. A recent review by the UK Scientific Advisory Group for Emergencies modelling group found limited data regarding the effectiveness of such devices[6]. Here we present the first data providing evidence for the removal of SARS-CoV-2 and microbial bioaerosols from the air using portable air filters with UV sterilisation on a COVID-19 ward.

Methods

The study was conducted in two repurposed COVID-19 units in Addenbrooke's Hospital, Cambridge, UK. One area was a 'surge ward' (ward) managing patients requiring simple oxygen therapy or no respiratory support, the second was a 'surge ICU' (ICU) managing patients requiring invasive and non-invasive (non-invasive ventilation, high flow nasal oxygen) respiratory support. The ward was a fully occupied four-bedded bay (top left panel Fig. 1A). The ICU was fully occupied five-bedded bay, with a supra-capacity sixth occupied bed used in week 2 (top left panel Fig. 1B). Both units were passively ventilated, with 2-4 air-changes per hour at baseline.

In the ward we installed an AC1500 HEPA14/UV steriliser (Filtrex, Harlow, UK), in the ICU we installed a Medi 10 HEPA13/UV steriliser (Max Vac, Zurich, Switzerland). The air filters were placed in fixed positions before the initiation of the three-week study period (Fig. 1A/B), switched on at the beginning of week two and run continuously from Sunday to Sunday for 24 hours per day, providing approximately 5-10 room-volume filtrations per hour.

We performed a crossover evaluation, with the primary outcome being detection of SARS-CoV-2 RNA in the various size fractions of air samples. Air sampling was conducted using National Institute for Occupational Safety and Health (NIOSH) BC 251 two-stage cyclone aerosol samplers[7] (B Lindsley, CDC), operated in accordance with previous studies [7,8]. Air samplers were assembled daily with a control sampler left in a sealed bag. Samplers were placed adjacent to the air filter inlet and the other at approximately four meters from the filter and no closer than two meters to patients. In ICU two distant samplers were used, one mounted at head height and one at bed height. Samplers were operated on weekdays (0815hrs to 1415hrs) for three consecutive weeks. After sampling, samplers were disassembled using sterile technique. The samples were processed then stored at −80°C until analysis

Nucleic acids were extracted from each NIOSH sampler component (tubes containing large aerosols, medium aerosols, and filter). Methodological details including extractions, RT-qPCR for SARS-CoV-2 and high throughput qPCR assays for a range of bacterial, viral, and fungal pathogens are in the supplemental methods (organisms listed in supplemental table 1). Differences in numbers of pathogens detected with filters on and off were compared by Mann-Whitney U test, $p \leq 0.05$ was considered significant.

Results

From January 18th to February 5th beds in the ward and ICU were at 100% occupancy; 15 patients admitted to the ward and 14 admitted to the ICU over the sampling period. All patients were symptomatic and tested positive for SARS-CoV-2 RNA.

In the ward, during the first week whilst the air filter was inactive, we were able to detect SARS-CoV-2 on all sampling days; RNA was detected in both the medium (1-4μM) and the large (>4μM) particulate fractions (lower panel Fig. 1A). SARS-CoV-2 RNA was not detected in the small (<1μM) particulate filter. The air filter was run continuously in week 2; we were unable to detect SARS-CoV-2 RNA in any of the sampling fractions on any of the five testing days. We completed the study by

repeating the sampling with an inactive air filter. As in week one, we were able to detect SARS-CoV-2 RNA in the medium and the large particulate fractions on 3/5 days of sampling (a sample without tube size indicated tested positive on day 5) (lower panel Fig. 1A). SARS-CoV-2 RNA was not detected from the control sampler.

We subjected the extracted nucleic acid preparations to high-throughput qPCR to detect a range of viral, bacterial, and fungal targets. In week one, we detected nucleic acid from multiple viral, bacterial, and fungal pathogens on all sampling days (top middle and right panels Fig. 1A). In contrast, when the air filter was switched on, we detected yeast targets only on a single day, with a significant reduction (p=0.05) in microbial bioaerosols when the air filter was operational (Fig. 1A). Using this high-throughput approach, SARS-CoV-2 RNA was detected on 4/5 days tested in week 1 but was again absent in week 2. We were unable to generate multiplex data for week three due to sample degradation following SARS-CoV-2 RNA amplification.

In contrast to the ward, we found limited evidence of airborne SARS-CoV-2 in weeks one and three (filter off) but detected SARS-CoV-2 RNA in a single sample in the medium (1-4μM) particulates on week 2 (filter on) (lower panel Fig. 1B). This contrary result did not reflect a general lack of bioaerosols in the ICU, which demonstrated a comparable quantity and array of pathogen associated nucleic acids to that seen in the unfiltered ward air on week one (top middle and right panels Fig. 1B). Again, the use of the air filtration device significantly (p=0.05) reduced the microbial bioaerosols (Fig 1B); with only three organism types detected on two of the sampling days. SARS-CoV-2 RNA was only detected once during week one on the high throughput qPCR assay.

Discussion

Our study represents the first report of removal of airborne SARS-CoV-2 in a hospital environment using combined air filtration and UV sterilisation technology. Specifically, we provide evidence for the circulation of SARS-CoV-2 in a ward within airborne droplets of >1μM. Droplets of 1-4μM are

likely a key vehicle for SARS-CoV-2 transmission[9], as they remain airborne for a prolonged period and can deposit in the distal airways. Recent data has shown that exertional respiratory activity, such as that seen in patients with COVID-19, increases the release of 1-4 μM respiratory aerosols, relative to conventionally defined 'aerosol generating procedures' such as non-invasive respiratory support [10]. Patients in ICU are commonly at a later stage of disease, and may shed less virus as a result. These data are consistent with our observations, suggesting that aerosol precautions may be more important in conventional wards than in well defined 'aerosol risk areas'.

The sampling and detection of airborne viruses poses several technological challenges, and there remains no agreed standard for their use or interpretation[11]. However, the detection of SARS-CoV-2 RNA by RT-qPCR (albeit at a high C_T value), and the lack of detection during use of an air sterilisation system, adds to a growing body of evidence implicating the airborne transmission of SARS-CoV-2[1]. The detection of SARS-CoV-2 RNA in the air of a ward managing patients with COVID-19 intimates that this is a key mechanism by which healthcare professionals could become infected. The removal of airborne viral particles and other pathogens may help reduce the likelihood of hospital-acquired respiratory infections. This reduction may be by both decreasing the load of respirable particles and by removal of larger droplets that can facilitate fomite-associated spread[11]. The clearance of bioaerosol was not restricted to SARS-CoV-2. Although the impact of air filtration on nosocomial infection is uncertain[5,12], the broad range of pathogens removed in this study suggests potential for benefit beyond SARS-CoV-2.

This study has limitations. The evaluation was conducted in two rooms and there are no data defining the optimal air changes required to remove detectable pathogens with the specified devices, nor their impact in better ventilated facilities. Given the large volume of air within the room and the stability of viruses in the sampling fluid, it was predictable that the amount of SARS-CoV-2 detected would be minimal. However, negative results from the control sampler, and the striking but reversible effect of the air filtration devices, suggest these are not false positive detections and we cannot exclude the risk

of airborne infection. Future studies should examine whether air filtration devices have an impact on healthcare professional and patient focussed outcomes, including measuring infection/exposure as an endpoint, as well as assessing potential harm, such as noise, reduced ambient humidity or impact on delivery of care.

We were able to detect airborne SARS-CoV-2 RNA in a repurposed COVID-19 'surge ward' and found that air filtration can remove SARS-CoV-2 RNA below the limit of qPCR detection. SARS-CoV-2 was infrequently detected in the air of a 'surge ICU'; however, the device retained its ability to reduce microbial bioaerosols. Portable air filtration devices may mitigate the reduced availability of airborne infection isolation facilities when surges of COVID-19 patients overwhelm healthcare resources and improve safety of those at risk of exposure to respiratory pathogens such as SARS-CoV-2.

Author contributions

ACM conceptualisation, methodology, data analysis, writing-original draft

KS investigation, supervision, writing-review and editing

RB investigation, supervision, writing-review and editing

LK investigation, data analysis, supervision, writing-review and editing

MM investigation, writing-review and editing

EH investigation, data analysis, writing-review and editing

SF investigation, writing-review and editing

JD investigation, writing-review and editing

TO investigation, writing-review and editing

SBr investigation, writing-review and editing

IH investigation, writing-review and editing

AK investigation, writing-review and editing

AT investigation, writing-review and editing

PW conceptualisation, provision of resources, writing-review and editing

AF Provision of resources, writing-review and editing

GD conceptualisation, provision of resources, supervision, writing-review and editing

EG conceptualisation, supervision, investigation, writing-review and editing

TG conceptualisation, supervision, investigation, writing-review and editing

SB conceptualisation, provision of resources, supervision, data analysis, writing-original draft

VN conceptualisation, provision of resources, supervision, data analysis, writing-original draft

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The study was registered as a service evaluation with Cambridge University Hospitals NHS Foundation Trust (Service Evaluation Number PRN 9798).

Data sharing statement

Data for organisms detected by single plex PCR (SARS-CoV-2) and high throughput PCR (bioaersol) are included as supplemental data files.

Conflicts of interest

Vilas Navapurkar is the founder, Director, and shareholder of Cambridge Infection Diagnostics Ltd. Andrew Conway-Morris, Paul White, Gordon Dougan and Stephen Baker are members of the Scientific Advisory Board of Cambridge Infection Diagnostics Ltd. Theodore Gouliouris has received a research grant from Shionogi. R Andres Floto has received research grants and/or consultancy payments from GSK, AZ, Chiesi, Shionogi, Insmed, Thirty Technology. Effrossyni Gkrania-Klotsas has received a National Institute of Health Research Greenshoots Award

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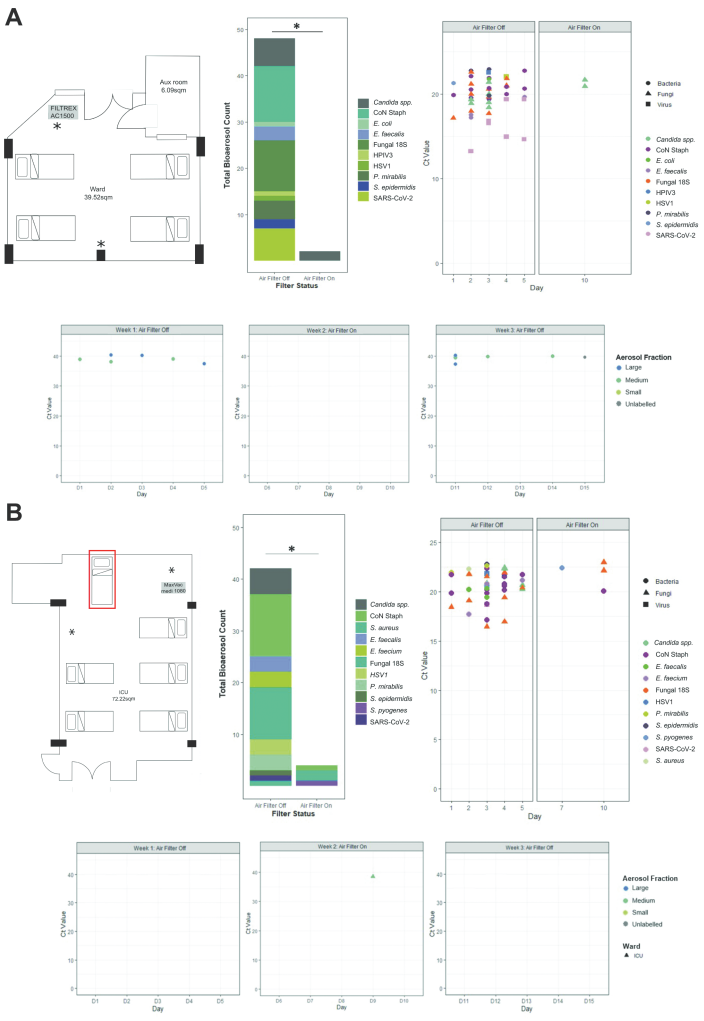
Figure 1. Bioaerosol detection in specific air sampler fractions over the three-week testing period on a 'surge' ward and 'surge' ICU.

A) Data from 'surge' ward. Panels depict; top left: Layout of the room on the 'surge' ward with four beds. The air filter was installed in the marked location and set to operate at 1,000 m^3/hour with a room volume of approximately 107 m^3. Top middle: Stacked bar chart showing collated total number of bioaerosol detections during weeks one (filter off) and two (filter on) *p=0.05 by Mann-Whitney U test. Top right: C_T values of detected pathogens by high-throughput qPCR when filter switch on and off. Bottom: C_T values for the single qPCR SARS-CoV-2 detection when filter switch on and off.

B) Data from 'surge' ICU. Panels depict; top left; Layout on the 'surge' ICU with six beds including the addition of a further supra-capacity bed to increase occupancy (labelled with red box). The air filter was installed in the marked location and set to operate at 1,000 m^3/hour with a room volume of approximately 195m^3. Top middle: Stacked bar chart showing collated total number of bioaerosol detections during weeks one (filter off) and two (filter on) *p=0.05 by Mann-Whitney U test. Top right: C_T values of detected pathogens by high-throughput qPCR when filter switch on and off. Bottom: C_T values for the single qPCR SARS-CoV-2 detection when filter switch on and off. N.B. variation in C_T values is a function of the microfluidics technology, and do not reflect higher bioaerosol burdens.


A
Aux room
6.09sqm
FILTREX
AC1500
Ward
39.52sqm
Total Bioaerosol Count
Filter Status
Air Filter Off
Air Filter On
Candida spp.
CoN Staph
E. coli
E. faecalis
Fungal 18S
HPIV3
HSV1
P. mirabilis
S. epidermidis
SARS-CoV-2
CT Value
Day
Bacteria
Fungi
Virus
Week 1: Air Filter Off
Week 2: Air Filter On
Week 3: Air Filter Off
Aerosol Fraction
Large
Medium
Small
Unlabelled
B
ICU
72.22sqm
S. aureus
E. faecium
S. pyogenes
Ward
ICU

Supplemental methods for "The removal of airborne SARS-CoV-2 and other microbial bioaerosols by air filtration on COVID-19 surge units"

Setting

The study was conducted in two repurposed COVID-19 units in Addenbrooke's Hospital, Cambridge, UK in January/February 2021 when the alpha variant (lineage B1.1.7) comprised >80% of circulating SARS-CoV-2 [S1].

Air changes in wards

Both the room in the 'surge' ward and 'surge' ICU were passively ventilated, without forced air changes.

Air filtration devices

The devices used were a AC1500 HEPA14/UV steriliser (Filtrex, Harlow, UK), whilst in the ICU we installed a Medi 10 HEPA13/UV steriliser (Max Vac, Zurich, Switzerland). The filter system has three stage particulate system: a coarse panel pre-filter, a secondary V-flow filter (ePM1=80%), and a HEPA filter, tested to EN1822 standards and >99.99% efficient at removing 0.3-micron particles. The filters are consistently exposed to 253nm UV-C lamps, certified to be 100% effective in removing microbiological agents. The units are certified to supply ISO5-EN ISO 14644 Cleanroom standard air (Class 100 US FED 209E). As the devices do not meet medical device electrical safety standards (EN60601) they were operated at a distance of ≥1.5metres from any patient.

National Institute for Occupational Safety and Health (NIOSH) BC 251 two-stage cyclone aerosol samplers

Each sampler collects large (>4 μM) particles into a 15 mL centrifuge tube, medium (1–4 μM) particles into a 1.5 mL centrifuge tube, and small (<1 μM) particles in a 37-mm diameter, polytetrafluoroethylene filter with 3-μm pores[S2]. Once sampling was complete samplers were disassembled using sterile technique and the filter papers were transferred to 15 ml Falcon tubes . The

pump flow rate was set at 3.5 L of air min^{-1}, using a flow calibrator and sampling duration set at six hours (collecting a total of 1,260 L/day), following criteria from previous studies demonstrating the capture of airborne viruses for RT-PCR detection[S3-7].

Nucleic acid extraction and polymerase chain reactions (PCR)

To facilitate solubilisation of nucleic acids, tubes were left on a tube rotator overnight at 4°C in lysis buffer containing 4M guanidine thiocyanate and 0.5% β-mercaptoethanol. After overnight solubilisation, all lysis buffer was removed from tubes and the extraction completed as described by Sridhar *et al*[S8]. All samples were eluted in 100 µl nuclease-free water and stored at -80°C until required for qPCR.

SARS-CoV-2 PCR

SARS-CoV-2 was detected in samples using the primers and method described previously[S8] . Briefly, 5 µl of the nucleic acid extract was combined with 20 µl master mix (12.5 µl 2X Luna Universal Probe One-Step reaction mix, 0.5 µl Wu forward and reverse primers (20 pmoles/µl), 0.3 µl Wu FAM-MGB probe (10 pmoles/µl), 0.5 µl MS2 forward and reverse primers (10 pmoles/µl), 0.3 µl MS2 ROX probe (10 pmoles/µl), 1 µl Luna WarmStart RT Enzyme Mix (New England Biolabs, Hitchin, UK) and 3.9 µl nuclease-free water) in a 96-well plate. Reactions were then run on the QuantStudio 7 Flex real-time PCR system (Thermofisher Scientific, Waltham, MA, USA) using the following cycling conditions: 2 minutes at 25°C, 15 minutes at 50°C (reverse transcription), 2 minutes at 90°C and then 45 cycles of 3 seconds at 95°C followed by 30 seconds at 60°C.

Bioaerosol high-throughput qPCR

Other pathogens were detected using a BioMark HD qPCR system (Fluidigm, Cambridge, UK). To prepare individual 10X assays for the BioMark HD qPCR, 2.5 µl of each forward and reverse primer pair (100 µM), was combined with 25 µl of 2X Assay Loading Reagent and 22.5 µl of TE buffer to a final primer concentration of 500nM. Microbial targets are listed in Table S1. Pooled assays for pre-amplification were produced by combining 1µl of all primer pairs and diluting to a final volume of

200 µl in TE buffer (Invitrogen, Thermofisher Scientific) to give a final primer concentration of 500 nM. Stock solutions of the pooled and individual assay mixtures were stored at −20°C.

4µl of nucleic acid extract was first reverse-transcribed using Fluidigm Reverse Transcriptase as per manufacturer instructions. Pre-amplification of cDNA was then performed to minimise sampling bias, using the Fluidigm PreAmp Master Mix Kit. 1.25 µl of reverse transcribed samples were then combined with 2.5 µl 2X PreAmp Master Mix, 0.5 µl pooled primers (500nM), 0.75 µl and nuclease-free water. Reactions were then run using cycling conditions of 95 °C for 10 minutes, followed by 17 cycles of 95 °C for 15 seconds and 60 °C for 2 minutes, and a final hold at 4 °C. Finally, samples underwent exonuclease I (Exo-I) (NEB) treatment to degrade any remaining single stranded DNA in accordance with manufacturer's instructions, before dilution 1:5 with TE buffer.

Samples were prepared for IFC (integrated fluidics circuit) loading as per manufacturer's instructions, with 2.5 µl of 2× SsoFast™ EvaGreen® Supermix Low ROX (BioRad, Watford, UK) and 0.25 µl of 20× DNA Binding Dye Sample Loading Reagent combined with 2.25 µl of the Exo-I treated samples. 5 µl of each assay mix (see above) and sample mix was loaded into the suitable IFC inlets and the IFC was loaded using the Fluidigm Juno. Once complete, the IFC was moved to the BioMark HD for qPCR using the pre-programmed thermal protocol: GE Fast 96x96 PCR+Melt v2.pcl.
Preliminary thresholding of the amplification data was completed using the Fluidigm Real-Time PCR Analysis Software, before raw data was exported to R (RStudio, Boston, USA) to apply manually defined melting curve peak thresholds. Positive samples were determined to be those with Ct values <= 23 and with melt curves within the previously determined range for that assay target.

Statistical analyses

Differences in the number of pathogens detected when air filter was on and off were compared by Mann-Whitney U-test. Statistical significance was inferred when p values were ≤ 0.05. Statistical testing and graphs generation were conducted in R studio.

Supplemental Table 1. Bacterial, fungal, and viral targets which formed the targets of the microbial bioaerosol high-thoughput qPCR*.

Bacteria	**Mycobacteria**	**Atypical bacteria**	**Fungi**	**Viruses**
Acinetobacter baumannii	*Mycobacterium tuberculosis*	*Chlamydia pneumoniae*	*Aspergillus fumigatus*	Adenovirus
Bordetella pertussis	*Mycobacterium* spp	*Chlamydia psittaci*	*Aspergillus* spp	Bocavirus
Bordetella parapertussis		*Coxiella burnetii*	*Candida* spp	HCoV 229E
Citrobacter spp		*Legionella pneumophila*	Fungal ribosomal 18S	HCoV NL63
Corynebacterium diphtheriae		*Legionella* spp		HCoV OC43
Escherichia coli		*Mycoplasma pneumoniae*		HCoV HKU1
Enterococcus faecium		*Leptospira* spp		Cytomegalovirus
Enterococcus faecalis				Epstein-Barr virus
Enterococcus sp				Enterovirus
Elizabethkingia meningoseptica				Herpes Simplex virus
Haemophilus influenzae				Influenza A virus
Klebsiella pneumoniae				Influenza B virus
Moraxella catarrhalis				Human Metapneumovirus
Morganella morganii				Measles morbillivirus
Neisseria meningitidis				Mumps virus
Proteus mirabilis				Parainfluenza
Pseudomonas aeruginosa				Parechovirus
Serratia marcescens				Rhinovirus
Staphylococcus aureus				Respiratory syncytial virus
Staphylococcus epidermidis				Rubella virus
Coagulase negative staphylococci				SARS-CoV-2
Stenotrophomonas maltophilia				Varicella zoster virus
Streptococcus pneumoniae				
Streptococcus pyogenes				

*Species were selected for their known respiratory pathogenicity or frequency as agents of hospital-acquired infection. HCoV human corona virus, SARS-CoV-2 severe acute respiratory syndrome coronavirus 2. Loading control was with bacteriophage MS2. (Primer sequences available on request)

Data availability.

qPCR and high throughput PCR results are contained as supplemental spreadsheets labelled SARS_AIR_qPCR and Fluidigm_Air_Raw1 respectively. A data dictionary is included in the supplemental section below.

Data dictionary

File: Fluidigm_Air_Raw1

File refers to high-throughput PCR obtained from Biomark HD device

Sample.Name – sample identifier: unit, sample number, day
Day – day of sampling
Day_number -day of sampling as number
Filter_location -near: close to air filter, away: away from filter, away_1: away from filter (bed height, ICU only), away_1.7:away from filter(head height, ICU only).
Week -week of evaluation (1, 2 or 3)
Filter_status -off:air filter present but not operational, on:air filter present and operational.
Unit -location of sampler: Ward: ward, ICU: ICU, Control:sampler assembled and placed in sealed bag.
Aerosol_Fraction- Large (>4μM), medium (1-4μM), small (<1μM)
Ct.Value-Cycles to threshold value
Pathogen- name of pathogen identified
Classification- type of pathogen identified
Interpretation- positive:appropriate melt dynamics, negative:inappropriate melt dynamics (where Ct and pathogen indicated) or nothing detected, failed:failure of internal QC

File: SARS_AIR_qPCR

Sample.Name – sample identifier: unit, sample number, day
Day_number -day of sampling as number
Unit -location of sampler: Ward: ward, ICU: ICU Control:sampler assembled and placed in sealed bag.
Filter_Location-off:air filter present but not operational, on:air filter present and operational.
Aerosol_Fraction- Large (>4μM), medium (1-4μM), small (<1μM)
Ct.Value-Cycles to threshold value
Week- week of evaluation (1, 2 or 3)
Filter status-off:air filter present but not operational, on:air filter present and operational.
Interpretation- positive:appropriate melt dynamics, negative:inappropriate melt dynamics (where Ct and pathogen indicated) or nothing detected, failed:failure of internal QC

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