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Situation, Background, Assessment, Recommendation (SBAR)

Cardiac Heater Cooler Units (HCUs) Air Sampling in the Management of HCUs

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Antimicrobial Resistance & Healthcare Associated Infection (ARHAI) Scotland
An NHS Scotland Assure Service, part of Public Services Delivery Scotland

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1 Situation

Current NHS Scotland guidance for the decontamination and testing of cardiac heater cooler units (HCUs), first published in 2019 and updated in 2025, recommends routine fortnightly air sampling of each HCU for detection of *Mycobacterium* species. In addition, positive water results for *Mycobacterium chimaera* or other mycobacterial species currently trigger air sampling as part of the investigation process.[1]

Following stakeholder feedback regarding the practical application of routine air sampling and challenges in interpreting results, ARHAI Scotland commissioned a rapid review of the literature to assess the current evidence base and non-UK guidance relating to the role of environmental sampling (including air sampling) in HCU management.

The rapid review identified evidence that does not fully support routine air sampling as currently described in Scottish guidance. However, the review also identified situations where air sampling remains a potentially valuable investigation tool. The findings of the rapid review indicate that the role of routine air sampling within current Scottish guidance requires further consideration. ARHAI Scotland will use these findings to inform interim advice for NHS boards and support future review and updating of national HCU guidance.

2 Background

HCUs are used during cardiothoracic surgery to regulate patient temperature. Although HCU water does not directly contact the patient, contamination of HCUs with non-tuberculous mycobacteria (NTM), particularly *Mycobacterium chimaera*, has been associated with serious invasive infections following cardiac surgery.

The rapid review included:

- 14 national and international guidance documents [2-15]
- three field safety notices[16-18]
- one manufacturer's instruction document[19]

- 19 primary studies encompassing outbreak investigations, surveillance studies and experimental research[20-38]

Key findings were:

Routine air sampling

- No identified national or international guidance document explicitly recommends routine air sampling of HCUs.
- Three guidance documents specifically recommend against routine microbiological testing because of concerns regarding lack of standardisation, lack of validation and the potential for false negative results.[5,7,13]

Air sampling versus water sampling

- Four identified outbreak investigations reported lower recovery of microorganisms from air samples than from HCU water samples.[20,26,27,30]
- Surveillance studies produced mixed findings, with two reporting lower recovery from air than water samples.[29, 33]
- Across primary evidence, water sampling was consistently found to be a sensitive indicator of HCU contamination and was generally more informative than air sampling for detecting contamination of HCUs. [20,23,24,27]
- Guidance from outside the UK and one field safety notice discuss water sampling alone [3,19] or in conjunction with air sampling.[2]

Potential value of air sampling

- Primary evidence demonstrated that air sampling may be useful in specific circumstances, including demonstrating aerosolisation of contaminants from HCUs, supporting risk assessment where aerosol transmission is a concern, investigating suspected HCU-associated outbreaks, assessing the impact of device defects or maintenance issues, and demonstrating relatedness between environmental and clinical isolates where advanced molecular methods are available.[20,26,27,30,33,35,36,38]

Device design and aerosolisation

- Several studies demonstrated that contamination was detected in air samples only when units were operating and that leakage from gaps, breaches or

design features could increase aerosol release. Air contamination was reduced when defects were remediated or when engineering controls were implemented.[20,26,27,33,35,38]

Overall, the evidence suggests that while air sampling can provide important information regarding aerosolisation, it is not more effective than water sampling for identifying HCU contamination.[20,26,27,29,33]

3 Assessment

ARHAI Scotland considers that the findings of the rapid review do not support the routine fortnightly air sampling of HCUs currently recommended in NHS Scotland guidance. The review identified:

- a lack of internationally standardised and validated air sampling methodologies
- variable yield and sensitivity compared with water sampling
- difficulties interpreting negative results
- limited evidence that routine air sampling provides additional information beyond water sampling
- significant resource implications associated with testing and prolonged culture periods

However, the evidence does support a role for targeted air sampling as part of a risk-based investigation strategy. In particular, air sampling may provide useful information:

- when an HCU water sample is positive for *Mycobacterium chimaera* or another NTM and there is a need to determine whether aerosolisation is occurring
- where there is suspicion of device contamination associated with maintenance failures, visible defects, leaks or damage
- where contamination persists despite remediation measures
- during outbreak or incident investigations requiring further assessment of potential patient exposure
- where air sampling findings would directly influence risk assessment, mitigation measures or patient management decisions

The evidence therefore supports a move from routine air sampling towards a targeted, proportionate and risk-based approach.

4 Recommendations

The following recommendations are proposed by ARHAI Scotland:

Operational recommendations for NHS Boards

1. NHS Scotland boards should continue to undertake HCU water sampling in accordance with current national guidance, recognising that current evidence indicates that water sampling is at least as effective as air sampling for identifying HCU contamination and remains a key component of environmental monitoring.[20,26,27,29,33]
2. Routine scheduled air sampling of all HCUs should no longer be regarded as a standard monitoring requirement.[5,7,13]
3. Air sampling should be considered as part of a targeted, risk-based investigation strategy where findings are expected to support risk assessment, incident management or decision making.[2,5,7,20,26,27,30,33,35,38]
4. Air sampling results should be considered alongside:
   - water sample results
   - device maintenance records
   - engineering and environmental assessments
   - microbiological and epidemiological findings
   - clinical risk assessments

   Air sampling should not be used in isolation to determine device safety or patient risk and should be interpreted in the context of wider environmental, engineering, microbiological, epidemiological and clinical assessment.[5,13,20,26,27,33]
5. Where positive HCU water results, positive air results, or concerns regarding aerosolisation are identified, NHS boards should continue to report incidents to ARHAI Scotland via the Outbreak Reporting Tool (ORT), the Incident Reporting and Investigation Centre (IRIC) and undertake local multidisciplinary risk assessment involving infection prevention and control, microbiology, cardiothoracic services, estates and medical physics teams.

6. Pending a full review of national guidance, NHS boards should continue to seek specialist advice where concerns regarding HCU contamination or aerosolisation arise.

ARHAI Scotland recommendations

1. The current NHS Scotland HCU guidance should be updated, as an interim measure, to reflect the above recommendations.
2. ARHAI Scotland should consider the findings of this rapid review as part of future review and development of national guidance relating to HCU management.

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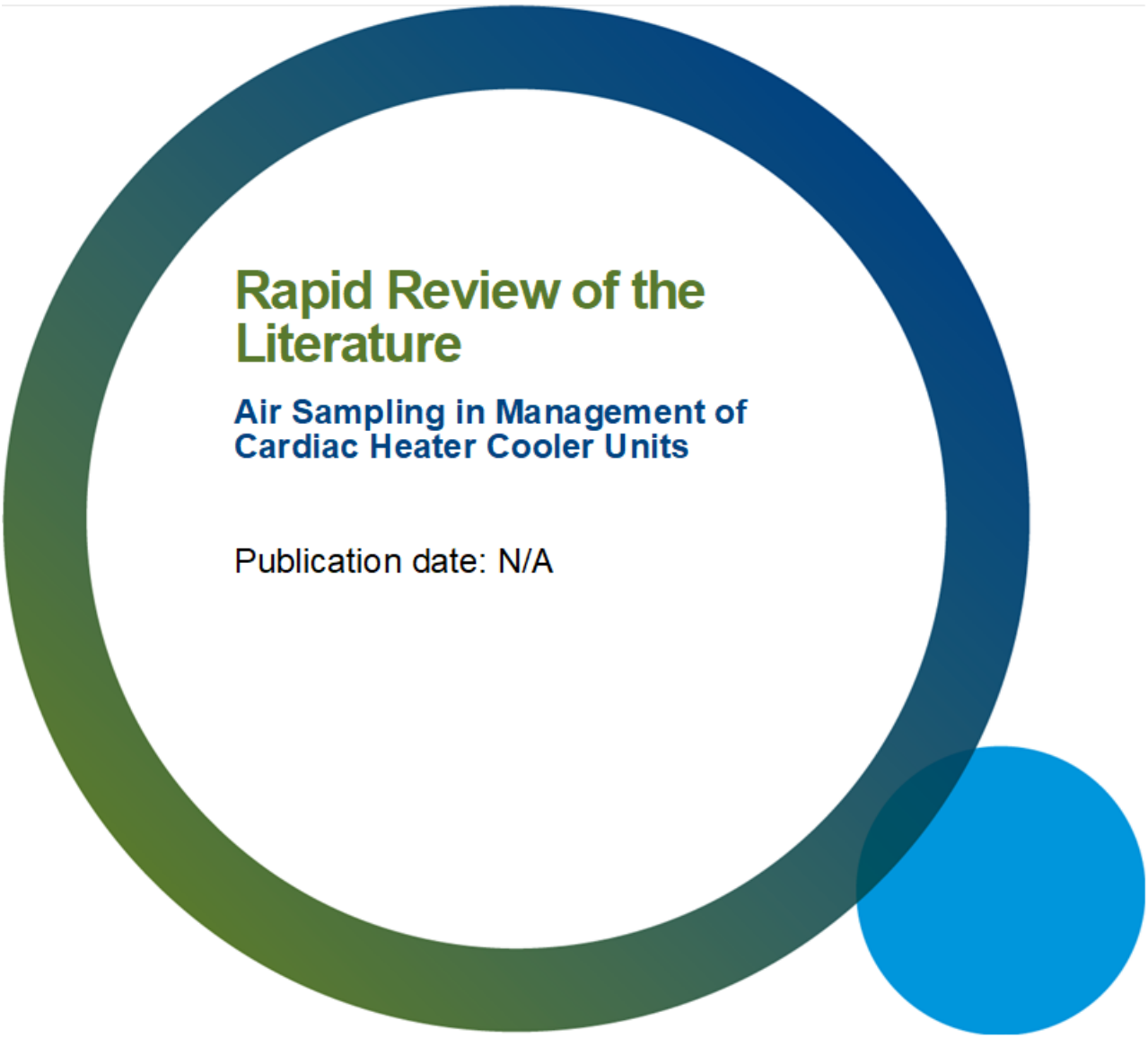
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Appendix 1 – Rapid review of literature: Air sampling in management of cardiac heater coolers units



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1. Introduction

In 2015, a global outbreak of *Mycobacterium chimaera* linked to heater-cooler units (HCUs) used during cardiac surgery was identified, which prompted changes to HCU practices.[1] A large volume of primary literature has been published on the infection risks associated with exposure to cardiac HCUs, indicating that these units may become contaminated at the site of manufacture, or within clinical settings.[1] In 2024, the UK Health Security Agency (UKHSA) reported findings from a retrospective study which identified 58 cases of *M. chimaera* related to HCUs used in open heart surgeries in the UK between 2006 and 2017. This study described long intervals between surgery and diagnosis (median = 24 to 26 months) and poor prognoses for affected patients (44 deaths).[2]

Scottish guidance, first published in 2019, advises routine air sampling (fortnightly) for *Mycobacterium* species (spp.).[3] The aim of this rapid review is to assess the extant scientific literature and guidance regarding the role of air sampling in the management of cardiac HCUs in health and care settings.

2. Objectives

The following research objective was considered:

- What is the role of air sampling in the management of heater cooler units?

3. Methodology

A rapid review of the literature was performed using a tailored search strategy (**Appendix 1**). The academic databases Medline and Embase were searched on 08 December 2025 to retrieve relevant literature published from the year 2010 onwards. Additional hand searching, and a grey literature search using the terms “heater cooler”, “cardi” and “cardiac heater cooler”, were conducted.

Title and abstract screening was conducted by one reviewer with a 20% check by a second reviewer. The second screen was conducted by two reviewers. As this was a rapid review, evidence was not formally graded with the use of an appraisal tool, however, where appropriate, the SIGN 50 principles were applied to critically analyse the evidence base.

To investigate the role of air sampling in real-world scenarios, the following primary studies were included:

- Outbreak reports where HCUs were investigated as a potential source

- Studies undertaking HCU sampling, including for the purposes of environmental surveillance

Outbreak reports which did not provide sufficient detail on the infection prevention and control measures implemented were excluded (see **Appendix 2**).

4. Results

4.1. What is the role of air sampling in the management of heater cooler units?

Following screening and data extraction of documents identified in targeted database and grey literature searches (**Appendix 3**), thirty-seven documents were included to address this research question.

4.1.1 Extant guidance

Fourteen guidance documents were included, six of which focus on *M. chimaera*,[4-9] two on *Mycobacterium* species,[10, 11] one on slow-growing *Mycobacteria*,[12] four on non-tuberculous *Mycobacteria* (NTM)[13-16] and one on *Legionella*.[17] Of the included guidance, four are from the UK,[8, 10-12] four from the US,[13, 14, 16, 17] two from Australia,[4, 9] two from the EU/EEA,[5, 6] one from Canada[15] and one is international guidance.[7] One guidance document reports use of an evidence review and expert consensus to develop recommendations,[7] three guidance documents report development by or in collaboration with experts,[5, 6, 15] and the remainder provide no clear methodology (n = 10).[4, 8-14, 16, 17] Most of the included guidance was published between 2015 and 2019 (n = 9)[4-6, 8, 10-12, 14, 15] with only one of these guidance documents reporting an update since 2020.[4]

In addition to the 14 guidance documents, three field safety notices (FSN)[18-20] and one manufacturers' instructions document[21] were also included, all of which are applicable to LivaNova HCU devices[18, 19, 21] (previously Sorin).[20]

The risk of healthcare associated infection from HCUs is widely acknowledged in extant guidance. Although most of these guidance documents refer to NTM,[4, 6-11, 13-16] the US Centers for Disease Control and Prevention (CDC) also recognise the risk for *Legionella* contamination of these devices.[17] Two included FSNs from LivaNova discuss design features which negate aerosolisation risks from their HCUs.[18, 19] The 2017 FSN for LivaNova Heater Cooler 3T devices announced a retrofit to prevent aerosol dispersion in the operating room, superseding an FSN from 2016 advising directing HCU exhausts away from the patient.[18] A further FSN for LivaNova

devices was published in 2019 announcing a device upgrade (vacuum and sealing design) to address air vent valve leakage amongst other issues.[19]

Nine of the included guidance documents provide non-specific guidance relating to HCU management. Four guidance documents discuss risk assessment of HCUs broadly,[8, 10, 12, 14] while seven documents suggest adherence with device manufacturers' instructions regarding maintenance of HCUs, including environmental sampling.[4, 6-8, 13, 14, 17]

Routine air sampling

Routine sampling is not explicitly recommended in any of the included guidance documents. Three guidance documents specifically advise against routine air sampling, highlighting issues with microbiological testing for these devices.[7, 9, 15] The International Society of Cardiovascular Infectious Diseases Guidelines for *M. chimaera* Infection (2020) and Government of Canada guidance (2017) describe limitations of routine microbiological screening associated with HCUs, including a lack of standardisation and validation of sampling and testing methods. Authors outline that this increases the risk of false negative results from device testing for *Mycobacteria chimaera*.[7, 15] Moreover, Australian Government guidance states that risk of infection from aersolisation of contaminated particles from HCUs is very low "based on the currently available data",[4] although it is not clear to which data they are referring.

Other indications for air sampling

Seven of the included guidance documents recommend adherence with device manufacturers' instructions which include indications for environmental sampling.[4, 6-8, 13, 14, 17]

Five guidance documents define indications for HCU-associated air sampling more specifically.[4, 7, 9, 12, 15] Australian Government guidance (2019) recommends air (and water) sampling when contamination of HCUs is suspected.[4] Examples of contamination indicators include discolouration of fluid lines and circuits.[4] Moreover, guidance from the Australian Public Health Laboratory Network suggests that air sampling may be considered "based on local considerations".[9] In addition, the FSN for Sorin Group perfusion system Heater Cooler 1T and Heater Cooler 3T devices (2015) recommend that if the HCU has not been maintained according to manufacturers' instructions, bi-weekly microbiological air samples for NTMs should be conducted.[20]

Two documents do not discuss indications for air sampling but do report routine sampling of other sites. The European Centre for Disease Prevention and Control (ECDC) only recommend

environmental sampling of water (outlets, contained within clinical equipment) and sterile medicated fluids,[5] in association with HCU sampling, to monitor control measures.[6] Moreover, LivaNova operating instructions for the Heater Cooler System 3T recommended regular water sampling to determine disinfection and water preservation efficacy, and to support investigations for external contamination sources.[21] Neither this document or any included FSNs provide any recommendation on air sampling, including its frequency.[18-21] It is not clear if routine air sampling is not recommended by all HCU manufacturers, as instructions for HCU devices produced by other manufacturers were not publicly available on manufacturer's websites.

Methods for air sampling

Two guidance documents, from the ECDC and Public Health England (PHE), provide specific protocols for HCU-associated environmental sampling (air and water) for isolation of slow growing *Mycobacteria*[12] and *Mycobacteria chimaera*.[5] These methods are consistent, although PHE recommend impaction sampling whereas ECDC recommend surface impaction sampling, and PHE recommend sampling a higher volume of air (see Table 1 for detailed methods), with the caveat that sampling over and above these volumes may be carried out "at a centre's discretion" so long as it is recorded.[12] In addition, the ECDC cite PHE guidance regarding how to process air samples.[5]

Table 1. Air sampling methods for HCUs described in extant guidance (n=2)

	ECDC (2015)[5]	PHE (2016)[12]
Organism	*Mycobacteria chimaera*	Slow growing *Mycobacteria*
Passive or active air sampling	Not specified	Active
Air sampling device	Surface impaction sampler	Impaction sampler
Volume of air	At least 250 to 500 litres	Total of $1m^3$ from each machine (equal to 1,000 litres), $0.5m^3$ from the front of the HCU and $0.5m^3$ from the rear

	ECDC (2015)[5]	PHE (2016)[12]
Culture methods	Standard-sized selective (antimicrobial agent-containing) 7H11 or 7H10 (Middlebrook) agar plates	Standard-sized 7H11 (Middlebrook) selective agar plates
Location of HCU during testing	Operating room	Operating theatres
HCU on or off during sampling	On (during cardiovascular surgery with cardiopulmonary bypass and during HCU operation)	On
Sampling location (from HCU)	0.3m from the front and rear of the HCU	0.3m from the front and rear of the HCU

Summary

In conclusion, extant guidance and (publicly available) manufacturers' instructions do not recommend routine air sampling of HCUs, with extant guidance citing lack of reliable microbiological methods and a very low risk of infection from aerosolisation of contaminated particles as rationale against routine testing. Some guidance documents suggest air and/ or water sampling may be carried out under specific circumstances, for example when device contamination is suspected or if the device has not been maintained (according to manufacturer instructions). Regarding air sampling method, extant guidance provides sampling protocols which encompass types of sampling (passive or active, air sampling device), volumes of air to be collected, culture methods, HCU location and function.[5, 12]

4.1.2 Primary studies

Nineteen primary studies were included which assessed the degree of contamination of HCUs and/ or the potential for HCU contaminants to be disseminated into the air during surgery and create a risk of infection for patients. The target organism for environmental sampling across the included studies was predominantly NTM,[22-25] specifically *Mycobacterium avium* complex (MAC) species,[26] *M. wolinskyi*,[27] *M. chimaera*,[24, 25, 28-31] and *M. abscessus*.[32, 33] Other microbiological testing targets included *Pseudomonas aeruginosa,*[34] coliform bacteria,[24, 34] Gram-negative

bacteria[35] and ‘microorganisms’ generally.[36] Key differences between the primary studies included their design: outbreak investigation in response to patient cases,[22, 27-29, 32, 33, 37] clinical and/ or environmental surveillance,[23-26, 30, 31, 34-36] or experimental sampling.[38-40]

Across the included evidence, HCU models, their age, and validation or maintenance practices differed. These characteristics are summarised in Table 2.

Table 2. Characteristics, validation and maintenance of tested HCUs in included primary literature (n=19)

	Characteristics	Validation process	Maintenance process
Sax et al. 2015[28]	LivaNova • Model, age and location not reported • HCU filled using filtered water	✗	**From 2012** onwards, aimed to align with updated manufacturer disinfection instructions for water type, water changes, and disinfection using hydrogen peroxide and sodium hypochlorite.
Dupont et al. 2016[27]	• HCU filled using tap water • Does not report HCU model, manufacturer, age or location	✗	✗
Garvey et al. 2016[36]	LivaNova 3T • In use since 2012 • Location not reported • HCU filled using filtered water	✗	**Prior to Medicines and Healthcare products Regulatory Agency (MHRA) notification**, followed manufacturer instructions for water type, water changes and disinfection using hydrogen peroxide and sodium hypochlorite. **Following MHRA notification**, two full decontamination cycles using sodium hypochlorite and increased frequency of water changes and disinfection. Following updates to manufacturer instructions, a change of disinfectant practices, including introduction of peracetic acid for disinfection.
Gotting et al. 2016[38]	HCUs (n=5), located outside of OR with door slightly open	✗	Aimed to comply with manufacturer instructions, but adherence was unclear.

	Characteristics	Validation process	Maintenance process
	• Does not report model, manufacturer or age • HCU filled using filtered water		Does not report maintenance of each HCU.
Schreiber et al. 2016[31]	LivaNova 3T (n=5), located in negative pressure ORs • Brand new • HCU filled using filtered water	Disinfection prior to first use	**Prior to April 2014:** compliance with manufacturer instructions on water changes and disinfection with sodium hypochlorite and hydrogen peroxide. **From mid-April 2014:** intensified cleaning and disinfection protocol. **December to September 2014:** repair of one HCU. **From February 2015:** change from sodium hypochlorite to peracetic acid and hydrogen peroxide as per manufacturer instructions and product availability.
Sommerstein et al. 2016[40]	LivaNova 3T (n=4), located in OR with ultraclean HEPA-filtered ventilation system • Commissioned in 2012, and used daily (n=1) • On loan from manufacturer since September 2016 (n=3) • HCU filled using filtered water	✘	✘

	Characteristics	Validation process	Maintenance process
Trudzinski et al. 2016[30]	Medos Deltastream HC, Maquet HU 35 and NovaLung NovaTherm • Age not reported • Located at patient bedside • HCU filled using filtered water	'Priming' of HCUs	×
Baker et al. 2017[32]	LivaNova 3T, located in ORs • Age not reported • Orientation adjusted during study period to direct exhaust away from surgical field • HCUs filled using unfiltered water, until awareness of manufacturer requirements, then changed to filtered tap water	×	**During phase 2 of outbreak**, practice review demonstrated non-compliance with manufacturer disinfection instructions for water type, water changes, and disinfection using hydrogen peroxide and sodium hypochlorite so protocol changed.
Chand et al. 2017[37]	LivaNova 3T, Maquet HCU 30 and Macquet HCU 40	×	×

	Characteristics	Validation process	Maintenance process
	• One decommissioned LivaNova 3T HCU in use since 2002 tested during simulated use (test chamber) • Location not reported for HCUs in use • Sterile water used to fill HCU for simulation study • Water used to fill HCU in practice not reported		
Garvey et al. 2017[39]	LivaNova 3T (n=1) • Age and location not reported • HCU filled using filtered water	✗	Tubing replaced by manufacturer annually due to biofilm growth. **Before 2015:** compliance with manufacturer instructions for replacement of water, disinfection using hydrogen peroxide, and sodium hypochlorite. **June to September 2015:** compliance with updated manufacturer instructions to increase disinfection frequency and inclusion of peracetic acid as part of disinfectant protocol. **September 2015 to June 2016:** compliance with updated manufacturer instructions on water exchange, and disinfection frequency and disinfection using hydrogen peroxide and peracetic acid with schedule to avoid degrading of internal components.
Lyman et al. 2017[22]	LivaNova 3T	Negative microbiological	Found non-compliance with manufacturer disinfection instructions.

	Characteristics	Validation process	Maintenance process
	• Acquired in 2009 (n=1) or 2012 (n=2) but since decommissioned • Location not reported • Used sterile water to fill HCUs, then changed to comply with manufacturer instructions (no further detail)	culture results from new HCUs prior to installation	No further detail reported.
Kuehl et al. 2018[29]	• LivaNova 3T and Maquet HCU 30 acquired in 2012. 3T™ used in first instance • Macquet HCU 40 acquired in 2016 to replace 3T™ • HCUs located in ORs with laminar airflow • Does not report type of water used to fill HCUs	HCU functioning prior to use	**For LivaNova 3T**, from July 2014, intensified disinfection protocol implemented using hydrogen peroxide and sodium hypochlorite. Protocol then updated to align with changes to manufacturer instructions, with inclusion of peracetic acid for disinfection and thermodisinfection. **For Maquet HCU 40**, regular water changes disinfection with tosylchloramide. Later change to increase frequency of disinfection.
Robertson et al. 2018[25]	LivaNova 3T (n=19) • Age, location and type of water not reported	✘	Hospitals ensured compliance with “biological equipment monitoring” and manufacturer instructions for disinfection of HCUs. Replacement of contaminated HCUs.

	Characteristics	Validation process	Maintenance process
Chan et al. 2019[24]	LivaNova 3T (n=3) and LivaNova 1T (n=4) • Age and location not reported • HCU filled using tap water	✗	Compliance with manufacturer instructions for disinfection using peracetic acid and hydrogen peroxide, disinfection of outer HCU surface using alcohol wipes, and water changes.
Ditommaso et al. 2020[34]	LivaNova 3T (n=17) and Maquet HCU 40 (n=12) • Age and location not reported • HCU filled using filtered water	✗	Compliance with manufacturer instructions for water changes, disinfection with hydrogen peroxide, sodium hypochlorite, tosychloramide and/ or peracetic acid, and removal of HCUs evidencing contamination.
Kaelin et al. 2020[23]	LivaNova 3T (n=5), located inside ORs, housed in steel housings with direct connection to OR exhaust • Purchased in 2014 • HCU filled using filtered water	Disinfection prior to first use	**Prior to April 2014:** disinfection with sodium hypochlorite and hydrogen peroxide and water changes. **From mid-April 2014:** changes to maintenance frequency. **From February 2015:** change of disinfectant product to peracetic acid and hydrogen peroxide as per manufacturer instructions.
Lecorche et al. 2021[26]	LisaNova (n=66), Maquet (n=3) and unknown brands (n=6)	✗	Compliance with manufacturer instructions for water type, disinfection with hydrogen peroxide, tosylchloramide, peracetic acid and/ or sodium hypochlorite, water changes and microbiological monitoring.

	Characteristics	Validation process	Maintenance process
	• Model, age and location not reported • Reports manufacturer requirements for HCU water (filtered water), but not if compliance met • Multisite study		Annual maintenance by manufacturers.
Seidelman et al. 2024[33]	CardioQuip MCH(i)-1000 • Age and location not reported • HCU filled using filtered water	×	×
Kuster et al. 2025[35]	Maquet HCU 40 (n=4), located in a separate room outside OR, apart from for emergency surgery • In use since 2016 • HCU filled using filtered water	×	Compliance with manufacturer instructions on water filter exchange and disinfection with chloramine-T.

× = no information provided

4.1.2.1 Outbreak reports

Six outbreak reports were included. These studies were conducted in France (n=1),[27] Switzerland (n=2)[28, 29] and the US (n=3)[22, 32, 33] and published between 2015 and 2024. Authors investigated environmental sources of patient infection, with a focus on HCUs due to:

- The identification of patient infections caused by NTM following surgeries requiring use of HCUs, and
- An awareness of the global outbreak of HCU-associated NTM infections following cardiac surgery.[22, 27-29, 32, 33]

Epidemiological evidence of transmission was not assessed in this review to evaluate the authors' hypothesised links between HCUs and patient infections or colonisations, nevertheless, where authors have reported epidemiological findings in relation to environmental contamination of HCUs, these are presented.

Air sampling; comparison with water samples

Across the six outbreak reports, four studies undertook air sampling next to HCU units in conjunction with sampling of HCU water.[22, 28, 29, 32] Additional sites sampled included HCU tank surfaces,[22] additional water sources,[22, 32] airflow,[29] and air velocity.[29] Air sampling methods and findings for these studies are overviewed in **Appendix 4**.

For four outbreak reports, air samples recovered fewer microorganisms than water samples.[22, 28, 29, 32] Conversely, Kuehl et al. found that *M. chimaera* was detected at higher percentages in air samples compared to water samples for one HCU model (LivaNova 3T) which had been in use for a prolonged period, and that air and water samples were closely related.[29] This study also reported significant differences in positivity of air samples across different HCU models ($p<0.01$), with the greatest proportion of contamination in LivaNova 3Ts acquired in 2012 compared to Maquet HCU 30 or HCU 40 models acquired in 2012 and 2016 respectively.[29]

Impact of HCU design on potential for Aerosolisation

Two outbreak reports by Sax et al. and Kuehl et al. provided detail on design features which authors outline have the potential to influence aerosolisation and vary across different HCU model:[28, 29]

- Degree of airtightness where overflow tubes, probes or stirring devices are present in or near water tanks
- Position and direction of ventilators relative to water tanks
- Velocity of air flow through compressors

Three outbreak reports evidenced that where air contamination with the target microorganism was identified, this occurred only in air samples taken adjacent to HCUs when turned on, and not when the unit was turned off.[22, 28, 29] Other sites which were positive for target organisms across three studies included: water fountains,[28, 32] showers,[28, 32] taps (including at scrub sinks),[22, 32] ice machines,[22, 32] and water basins.[32]

Across the identified outbreak reports, HCUs were sourced from LivaNova (n=4),[22, 28, 29, 32] Maquet (n=1),[29] and CardioQuip (n=1)[33] (**Table 2**). Five of these studies were published between 2015 and 2018,[22, 27-29, 32] in the period following identification of the global *M. chimaera* outbreak[1] and during which FSNs were regularly published on the risk of HCU aerosolisation and changes to design features (see section 0). Only two studies reported HCU manufacturer, model and age,[22, 29] and both of these studies investigated LivaNova 3T HCUs, to which design changes have been made to eliminate the potential for aerosolisation since their publication.[18, 19] It is unclear to what extent other HCU models have undergone design changes since publication of the identified outbreak reports.

Comparison with patient isolates

Three outbreak reports provided evidence implicating HCUs as a source of patient infection,[22, 28, 32] with hypothesised transmission via aerosolization of microorganisms by the HCU[28, 32] or leaking of the HCU with subsequent contamination of patients' blood.[28] Two studies demonstrated clustering of clinical and LivaNova 3T HCU-associated environmental isolates (air and water).[22, 32] Baker et al. did not report HCU age,[32] while Lyman et al. stated that their HCUs were acquired in 2009 and 2012.[22] Since publication of these outbreak reports, LivaNova have undertaken upgrades to their 3T HCUs with the aims to prevent aerosolisation of water (for more detail see section 0). A further outbreak report by Sax et al., published in 2015, found that air and water isolates from LivaNova HCUs (model not reported) were closely related and, despite not assessing relatedness to clinical samples, epidemiological evidence was suggestive of the HCU as a potential source of infection, although other environmental sites were also found to be contaminated.[28]

HCU water sampling only

Two outbreak reports were included where air sampling was not conducted as part of environmental investigation.[27, 33] These studies, published in 2016[27] and 2024,[33] sampled water from showers and sinks,[27] HCUs (tanks,[27] drains[33] and hoses[33]) and other water sources.[33] Additionally, Dupont et al. reported dry surface swabbing[27] and Seidelman et al. described smoke tracer experiments.[33] Both studies demonstrated that NTM species were present in water of HCUs,[27, 33] as well as taps[27, 33] and water-containing equipment.[33] An epidemiological association was demonstrated between CardioQuip MCH(i)-1000 HCUs (age not reported) and patient infections in the study by Seidelman et al,[33] and between taps and patient infections for both studies.[27, 33] Unfiltered tap water[28] and filtered tap water[34] was used to fill HCUs in these studies. Identification of NTMs in the healthcare environment (including within the HCU) was used to inform control measures and to assess their efficacy.[27, 33]

Summary

In summary, all four outbreak reports that utilised air sampling in proximity to HCUs also undertook water sampling of HCUs and both sources were found to be positive for NTMs in all four studies. In three studies, air samples recovered fewer organisms than HCU water samples; in one study organisms were greater in air samples compared to HCU water. All four studies reported either close relatedness of air and HCU water sample isolates or clustering of air and HCU water sample isolates. Studies (n=2) which did not conduct air sampling demonstrated that HCU water, other water sources, and surfaces were contaminated with NTMs. In one of these studies, isolates from the HCU water were clonally matched to patient isolates; this study utilised smoke testing to visualise the air flow from HCUs into the room.

4.1.2.2 Surveillance and experimental sampling studies

Ten surveillance studies from the UK,[36, 37] France,[26] Germany,[30] Italy,[34] Switzerland[23, 31, 35] Singapore[24] and Australia,[25] published between 2016 and 2025, investigated HCUs as environmental sources of contamination.[23-26, 30, 31, 34-37] Three of these studies did not report on the rationale for environmental sampling.[23, 26, 30] The remaining seven studies conducted surveillance to assess the efficacy of HCU disinfection practices,[36] as per the advice of manufacturers[34] or regulatory authorities,[35] or in response to identification of patient infections occurring regionally[25, 31] or being reported in published literature.[24, 37] Three of ten surveillance

studies provided evidence of relatedness between patient cases and environmental samples.[25, 26, 37]

Three experimental studies from the UK,[39] Germany[38] and Switzerland,[40] published between 2016 and 2017, were also included. These studies undertook HCU air[38, 40] and/ or water sampling[38, 39] for the following reasons: in response to evidence of HCU-associated patient infections in the literature,[38, 39] to explore HCU contamination,[38] to compare the efficacy of disinfection protocols[39] or to assess whether HCUs contaminate the surgical field.[40] None of these experimental studies provided evidence of relatedness between patient cases and environmental samples.

Seven surveillance or experimental studies undertook air sampling next to HCU units in conjunction with samples of HCU water,[23, 30, 31, 35, 37, 38] tap water,[30] other water sources,[37] and swabs of taps[35] and other surfaces.[30] Three of these studies further measured HCU particle emission (particle counts),[37, 38, 40] airflow,[37] assessed movement of smoke[38, 40] or conducted visual inspections of HCUs.[37] Air sampling methods and findings as per these studies are overviewed in **Appendix 4**.

Air sampling; comparison with water samples

Six studies undertook both air and HCU water sampling. Two surveillance studies indicated that HCU-associated air samples were less often positive than HCU water samples, although both successfully detected some of the NTM species that were identified in HCU water.[31, 35] Schreiber et al. found that despite only one NTM-positive air sample of a total 91, the *M. chelonae* isolated from the air sample was not found in water sampled from the same HCU.[31] Two studies demonstrated that air samples detected the same microorganisms as those found in HCU water samples,[37, 38] and for one of these studies air and water samples were positive in similar proportions.[37] Only Kuster et al. evidenced genetic relatedness between air, HCU water and tap swab isolates.[35] Two studies found that air and HCU water samples isolated different species of microorganisms.[23, 30] Kaelin et al. reported that *Mycobacteria* spp. isolated from air were distinct from species in HCU water samples, although not all species in water samples were reported.[23] Similarly, Trudzinski et al. found that air samples grew environmental moulds, Gram-positive and Gram-negative bacteria but not the target pathogen of interest, *M. chimaera,* which was isolated in water samples of all but one HCU.[30]

Impact of HCU design and placement

Chand et al. used smoke tracer and particle count measurements to evidence that holes and gaps identified in the older decommissioned LivaNova 3T HCU increased particles and altered the movement of air in and around the HCU.[37] For this decommissioned HCU, there was a higher load of *M. chimaera* and other organisms and total particles in the air adjacent to the HCU when the machine was turned on versus off ($p<0.01$), including at the site of holes and gaps prior to their sealing ($p<0.01$). These breaches to the HCU were identified only after removing its panels, which required manufacturer permission, thus would seemingly not be identified during regular maintenance of the machine.[37] Similarly, two experimental studies evidenced that air contamination (with *M. chimaera*[38] or particles[40]) was highest when HCUs were turned on, although this was not accompanied by statistical analysis findings in either case. These findings are in line with those of Kuster et al, who reported that air samples adjacent to HCUs were only positive when HCUs were functioning.[35] Sommerstein et al. indicated that positioning of HCU ventilation affected both particle counts at the surgical field, and whether smoke was or was not blown into the surgical field, providing evidence that facing HCUs away from the surgical field may be more beneficial in reducing exposure risk.[40] Further findings of this experimental study were that low numbers of *M. chimaera* colony forming units (CFUs) were present on settle plates (0 to 3 CFUs), with fewer CFUs at further distances from HCUs, providing weak evidence that HCU placement may affect air contamination. There was no further discussion of HCU design or placement in the included surveillance studies.

HCU water sampling only

Five surveillance studies[24-26, 34, 36] and one experimental study[39] only conducted sampling of water from HCU machines,[24, 26] including their circuits,[25, 34, 39] drains,[25] and incoming water.[36] Garvey et al. further dismantled a HCU unit, and found biofilm within HCU tubing.[36] The above studies found that HCUs tested positive for NTMs[34] including *M. chimaera*,[24-26, 36, 39] other *Mycobacteria ascessus* complex (MAC) species (*M. gordonae, M. paragordonae, M. chelonae, M. mucogenicum, M. avium* and *M. peregrinum*),[26] gram-negative bacteria,[36] *P. aeruginosa,*[24, 36, 39] *Paecilomyces* spp.[36] moulds[39] and coliforms.[24, 34] Five studies used HCU water sampling results to inform implementation of control measures,[24-26, 34, 36] and for four studies HCU contamination results were used to further evaluate the effectiveness of HCU disinfection following changes to protocols.[24, 34, 36, 39] None of these six studies conducted environmental sampling at any location other than HCUs.

Comparison with patient isolates

Three surveillance studies, published in 2017,[37] 2018[25] and 2021,[26] undertook surveillance of patient infections in addition to environmental surveillance.

Chand et al. undertook retrospective and prospective case finding to identify 18 and 2 probable cases of *M. chimaera* infection in England and Wales respectively.[37] A total of 24% air samples collected next to LivaNova 3T HCUs in practice were positive for mycobacteria, with five samples containing *M. chimaera*.[37] No mycobacteria were identified in the air adjacent to Maquet HCU 30 and HCU 40 machines. Concurrent analyses demonstrated that *M. chimaera* from clinical samples, a reference genome, and HCU environmental isolates were found to cluster closely, but the phylogenetic tree does not report whether any of these environmental samples were from the air. It is therefore unclear if air sampling was effective at identifying HCUs as a potential source or reservoir of infection for infected patients.[37] Two further studies undertook surveillance of patient infections,[25, 26] of which one found evidence of relatedness between clinical and HCU water isolates from HCUs.[26] Lecorche et al. reported that clinical case isolates from patients infected with *M. chimaera* were genetically related to 72% (n=26) of isolates from HCU water, across 61 healthcare facilities, although only one case patient had undergone an operation where a contaminated HCU was present.[26] These findings indicate that implicated HCUs (LivaNova of unknown model and age) may have caused or facilitated *M. chimaera* infection in this patient, and also demonstrated widespread contamination of HCUs in these facilities with a strain implicated in a previous Swiss outbreak.[26] As other environmental sampling was not undertaken, it is unclear if there were other sources or reservoirs of infection at these healthcare facilities.[26]

All other surveillance and experimental studies included did not assess environmental sources or reservoirs of patient infection or transmission routes of pathogens through the health and care environment.

Summary

Surveillance and experimental studies that compared HCU water and air samples demonstrated mixed results with some studies reporting detection of the same organisms in both sample types and others reporting detection of different organisms in HCU water and air samples. There was insufficient evidence regarding the impact of HCU design, however primary studies indicate that breaches or defects in units may create air leakage and that units are more likely to disperse particles when they are switched on (compared to off). Primary studies which did not

sample the air adjacent to HCUs indicated that sampling of HCU water and inspection of internal HCU machinery may identify microorganisms and/ or isolate species known to cause patient infection.

5 Conclusion

A large volume of primary and grey literature was included on the topic of HCU air sampling. Conclusions which may be drawn across the evidence base are as follows:

- Extant guidance (published 2015 to 2019) is consistent in reporting that HCU contamination has been and may continue to be associated with patient infections.
- Extant guidance does not recommend routine air sampling of HCUs:
    - Extant guidance recommends air and/ or water sampling where HCU contamination is suspected; four of the six included outbreak reports sampled the air as part of environmental investigation of patient infections.
- Primary literature reported alignment with manufacturer guidance for maintenance of HCUs, which complied with consistent advice of grey literature.
- Primary literature indicated that air samples were less or equally likely to identify HCU contamination than HCU water samples in most cases, with only one example whereby a microorganism was identified only in the air.
- In one outbreak study, isolates from the HCU water were clonally matched to patient isolates; this study utilised smoke testing to visualise the air flow from HCUs into the room. In two additional outbreak studies, isolates from both HCU water and air were clonally matched to patient isolates. Together, these findings indicate that HCUs may have been a source or reservoir of infection for outbreak cases; air samples (and smoke testing) was used to support the hypothesis that contaminated HCUs may have aerosolised microorganisms.
- Primary literature further evidenced that in the absence of air sampling, inspection of HCU internal machinery, or sampling of HCU water, other water sources and surfaces can be used to identify contamination.

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Appendices

Appendix 1: Search strategy

Embase <1974 to 2025 December 03>

Ovid MEDLINE(R) ALL <1946 to December 04, 2025>

Line	Search Term(s)	Result number
1	Heater-cooler*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]	567
2	Heater cooler.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]	562
3	1 or 2	567
4	(Infection or Incident or Outbreak or Contamination).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]	5,757,815
5	(Sampling or Environmental Sampling or Air sampling or Water sampling).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]	1,253,178
6	(infection control or Outbreak management).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]	183,484
7	4 or 5 or 6	6,844,053
8	3 and 7	396
9	limit 8 to yr="2010 -Current"	382
10	remove duplicates from 9	274

Appendix 2: Studies excluded at data extraction

The following studies had limited relevant conclusions for this rapid review:

Gravagna K, DiBiase L, Teal L et al. Outbreak Investigation of *Mycobacterium abscessus* Infections. South Med J. 2025 Aug;118(8):542-546. doi: 10.14423/SMJ.0000000000001864.

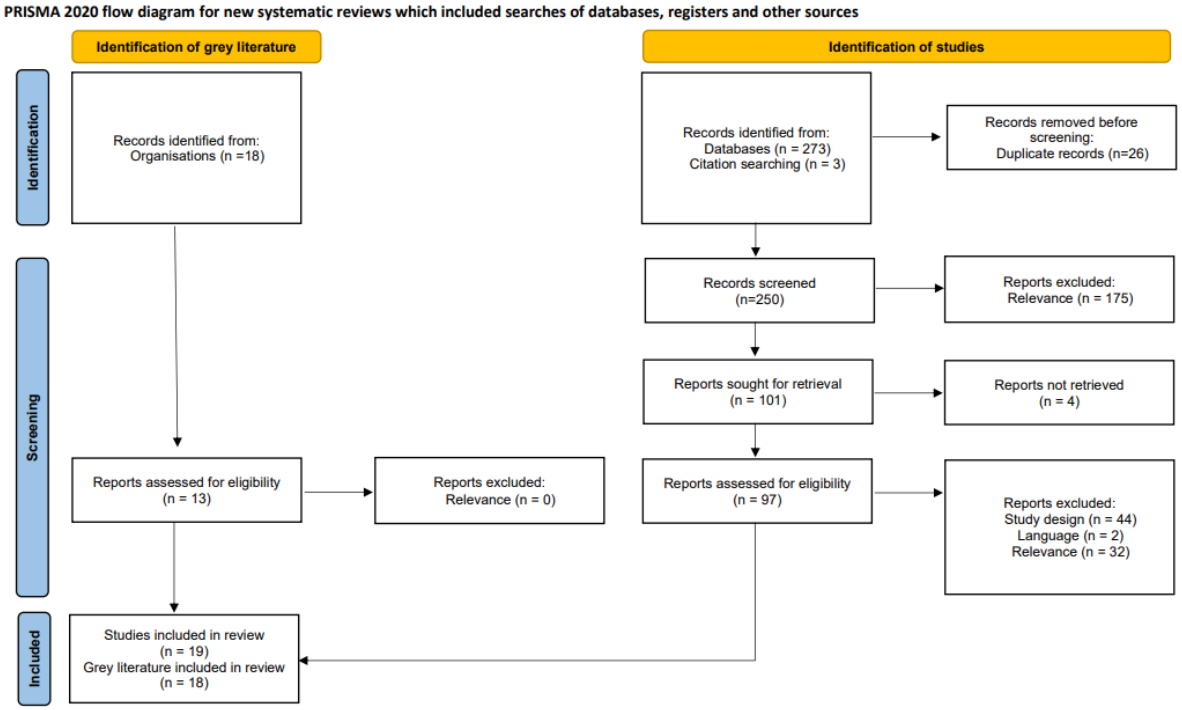
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Thomas S, Stevenson D, Otu AA et al. Microbial contamination of heater cooler units used in extracorporeal membrane oxygenation is not aerosolized into the environment: A single-center experience. Infect Control Hosp Epidemiol. 2020 Feb;41(2):242-244. doi: 10.1017/ice.2019.230.

Appendix 3: PRISMA Flow Diagram



Source: Page MJ, et al. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

Appendix 4: Air sampling protocol and findings from primary literature (n=11)

	Study design	Sampling protocol	Laboratory protocol	Findings
Sax et al. 2015[28]	**Outbreak report**	HCUs both on and off during sampling Air sampling undertaken in ORs, ICUs and wards (no ventilation parameters provided) Air impaction sampler (100l/min, 2.5 minutes)	Mycobacterial agar plates Incubation at 37°C for seven weeks or until positive RAPD-PCR to assess genetic relatedness	Air samples negative from March 2014 until Sep 2014 when *M. chimaera* identified in one HCU sample Air samples negative when HCUs were turned off No relatedness to clinical samples
Gotting et al. 2016[38]	**Experimental study**	Sampling while HCUs turned on and off Settle plates placed in front of HCU at distance of 1 to 3 metres, left open to the air for 4 hours Impaction air sampler (200l, 2 minutes) in area around the operating table, usual HCU located outside of OR with door slightly open	Mycobacterial agar plates Incubation at 37°C for 7 weeks Weekly monitoring Ziehl Neelsen stain in liquid media to confirm Mycobacteria 16s rRNA sequencing for identification of AFB 16S-23S rRNA ITS sequencing for identification of M. *intracellulare* *M. chimaera* confirmed by	*M. chimaera* detected in the exhaust air of all HCUs whilst turned on, but not when turned off *M. chimaera* detected at all locations

	Study design	Sampling protocol	Laboratory protocol	Findings
			external research centre No genetic relatedness assessment	
Schreiber et al. 2016	**Surveillance study**	HCUs located in negative pressure ORs Samples collected 2 to 3 metres from HCUs, while HCUs turned on, using an impaction air sampler (100l/min, 2.5 minutes)	Mycobacterial agar plates Incubation at 37°C for seven weeks or until positive 16s rRNA sequencing	98.9% (n=90) samples negative for Mycobacterial growth One sample positive for *M. chelonae*, despite corresponding water sample for this HCU negative for mycobacteria
Sommerstein et al. 2016[40]	**Experimental study**	HCU sampled when on, in OR with ultraclean HEPA-filtered ventilation system (room of size 2m x 2.4m), room airflow reported Quality check two months after smoke dispersal experiment *M. chimaera* settle plates in non-ventilated office, (dimensions 4m x 7m x 2.5m) with control room empty	Mycobacterial agar plates Incubation at 37°C for 7 weeks 16S rRNA sequencing to identify species No genetic relatedness assessment	Smoke dispersal experiments • HCU airflow away from operating field: smoke travelled in to and out of HCU ventilation as intended, then evacuated by OR exhaust • HCU airflow towards operating field: smoke blown away from HCU, reaching surgical field

	Study design	Sampling protocol	Laboratory protocol	Findings
		of HCU. Plates left open to the air for 4 hours, placed on the ground at 3 to 5 metres from HCU Smoke dispersal experiment: • 3 researchers present in the room • Smoke placed at vertical horizontal centre 0.2 metres from ventilation grid openings of the HCU facing the operating table • Experiments recorded using two cameras at eye-level at horizontal angle of 90° • Two scenarios, with airflow directed away or towards operating field Particle counts • Laser counter placed at the location of open chest surgical field 2 metres		after 10 seconds (0.15m/s) Particle counts • HCU off for five mins: 5.2 particles/min (range 1 - 12) • HCU on, airflow towards operating field for 5 mins: 139 particles min (range 62 - 212) • HCU on, airflow away from operating field for 4 min: 14.8 particles/min (range 5 - 24) Settle plates • 3m from HCU: 2 CFUs, M. chimaera • 5m from HCU: 1 CFU • 4m from HCU: 0 CFUs Controls: 0 CFUs

	Study design	Sampling protocol	Laboratory protocol	Findings
		below ventilation system ceiling outlet • Sampling opening pointing upwards Two scenarios, with airflow directed away or towards operating field		
Trudzinski et al. 2016	**Surveillance study**	Samples in patient rooms next to ECMO and HCU machinery, next to patients, and 2 to 3 metres from patients HCUs located at bedside HCUs turned on during sampling 100 to 200l samples over one minute	Mycobacterial media plates and liquid cultures Incubation for up to 8 weeks, checking for growth twice weekly Sample of *M. chimaera*- positive specimens sent to reference lab for 16S, ITS and *rpoβ* sequencing	All samples negative for mycobacteria or non-fermentative Gram-negative rods Low numbers of environmental Gram-positive and Gram-negative bacteria and environmental moulds isolated Does not report genetic relatedness despite typing
Baker et al. 2017[32]	**Outbreak report**	Does not report if HCUs on or off during sampling Sampling undertaken in ORs HCU orientation adjusted during the study period to	'Standard mycobacterial culture methods' Modification of protocol from a referenced study	No source of infection (HCUs) found until phase 2 of investigation, but air samples remained negative for *M. abscessus*

	Study design	Sampling protocol	Laboratory protocol	Findings
		direct exhaust away from surgical field	Subspecies identification of *M. abscessus* Genetic relatedness assessment via *erm41* and *rpoβ* sequencing, VNTRs and PFGE	70 patient isolates related to environmental isolates (including HCU air) forming two clones
Chand et al. 2017	**Surveillance study**	To simulate HCUs in practice, one decommissioned HCU located inside a test chamber simulating an operating room (HEPA-filtered air supply, 2 air changes per hour). Samples collected when HCU turned on and off: • Cyclone glass sampler (700l/min, 5 minutes) placed 50cm from floor and 30cm from front or rear of HCU • Aerodynamic particle sampler (5-minute intervals, detects size 0.5 to 20µm) positioned 2cm	Mycobacterial media or TSA Incubation at 30°C for five days (cyclone sampler) or at 35°C for up to six weeks Staining to identify mycobacteria Mycobacteria processed at reference laboratory Line probe assay to identify *M. intracellulare* Sequencing of ITS to identify *M. chimaera* WGS of *M. chimaera*, comparing isolates to an identified *M. chimaera* strain of known sequence	<u>Simulation study</u> Air samples significantly increased from HCU machine being off vs on ($p<0.01$) *M. chimaera* and other organisms identified Highest aerosol from the rear of the machine • Breaches in the tank covers, aerosol release from holes close to both circuit pipes, gap between sealing plate • Significant increase in particle counts of decommissioned HCU being off vs on at

	Study design	Sampling protocol	Laboratory protocol	Findings
		from obvious gaps and holes within HCU when side panels removed • Smoke tracer to visualise airflow Four-week investigation with 10 sampling events of HCUs in practice while turned on: • Air impaction sampler (1m^3, 100l/min) Liquid impinger at some locations	WGS of 13 representative MAC species strains Construction of phylogenetic trees	identified holes and gap ($p<0.01$) • Flow of particles from affected areas through rear cooling fan • Sealing gaps and holes significantly reduced particle counts ($p<0.01$) <u>HCUs in hospitals</u> Air samples near operating HCUs recovered mycobacteria in 24% (n=6) and *M. chimaera* in 5 samples No mycobacteria in air samples from Maquet HCU 30 or HCU 40 units • None of these units had visible gaps One tank breach observed
Lyman et al. 2017[22]	**Outbreak report**	Samples while HCUs on and off for simulation studies • Prior to turning on HCU	Sample processing by CDC laboratory AFB staining Genetic relatedness assessment via	Samples near HCU exhaust vents during simulation were positive for *M. chimaera* after 2, 3,

	Study design	Sampling protocol	Laboratory protocol	Findings
		• Intermittently over a five-hour period after turning on Not reported whether HCUs on or off for sampling in clinical practice 200l and 500l air collected per sampling event	MALDI-TOF, 16S and *rpoB* sequencing, PFGE and WGS	and 4 hours of HCU operation All other air samples during simulation, those adjacent to HCUs switched off, were negative for all NTM Isolates from all three *M. chimaera cases* and air highly related
Kuehl et al. 2018[29]	**Outbreak report**	Sampling while HCUs on and off, in ORs with laminar airflow 'Continuous' monitoring of HCU contamination over study period Mycobacterial air sampler (>160l collected) Approximately 1m from HCU rear Velocity measurements via anemometer held vertically to HCU exhaust at sides	Sample processing in accordance with Swiss national laboratory protocol Mycobacterial agar Incubation at 37°C for 7 weeks Genetic relatedness assessment via 'partial' 16S rRNA sequencing Did not assess relatedness of air and clinical isolates	Samples positive for *M. chimaera* initially from LivaNova 3T HCUs but not from Maquet HCU 30 HCUs No positive samples from any HCUs that were off After change in disinfection products to align with manufacturer instructions, and replacing the LivaNova 3T HCU with Maquet HCU 40 HCU, no more *M. chimaera* isolated from air

	Study design	Sampling protocol	Laboratory protocol	Findings
		Handheld smoke generator to visualise airflow		
Kaelin et al. 2020	**Surveillance study**	HCUs located inside ORs, housed in steel housings with direct connection to OR exhaust HCUs turned on during sampling Samples collected using air impaction sampler (100l/min, 2.5 minutes) 2 to 3 metres from HCUs	Mycobacterial agar plates 'Partial' 16S rRNA sequencing	2% (n=3) samples positive for rapidly-growing NTM NTM species identified: *M. mucogenicum, M. fortuitum* and *M. abscesses/chelonae* No isolation of *M. chimaera*
Kuster et al. 2025	**Surveillance study**	HCUs located in a separate room outside OR, apart from for emergency surgery Sampling while HCUs turned on and off Sampling performed intermittently, following initial detection of *M. chelonae* Air impaction sampler (250l, 2.5 minutes, 100l/min) 30cm from ground ("fan height") and 120cm from HCU	Mycobacterial agar plates Incubation at 37°C for 7 weeks in 5% carbon dioxide Short-read WGS of mycobacteria MLST and cgMLST Reference genome used to develop phylogenetic minimum spanning tree	8.7% (n=8) *M. chelonae-positive* samples over study period One sample positive for both *M. gordonae* and *M. chelonae* *M. chelonae* isolated from two samples, then a further sample 1.5 years later, from samples adjacent to running HCUs Air samples adjacent to HCUs turned off were all negative No positive samples from air next to

	Study design	Sampling protocol	Laboratory protocol	Findings
				HCUs turned off, or from samples in ORs Close relatedness of *M. chelonae* environmental isolates

AFB = Acid Fast *Bacillus*; CDC = US Centers for Disease Control and Prevention; cgMLST = core genome MultiLocus Sequence Typing; ECMO = extracorporeal membrane oxygenation; HEPA = High-efficiency particulate air; ITS = Internal Transcribed Spacer; MALDI-TOF = Matrix-Assisted Laser Desorption/Ionization - Time-of-Flight; MLST = Multi Locus Sequence Typing; NTM = non-tuberculous mycobacteria; OR = operating room; PFGE = Pulsed Field Gel Electrophoresis; RAPD-PCR = Random Amplified Polymorphic DNA Polymerase Chain Reaction; rRNA = ribosomal ribonucleic acid; VNTR = Variable Number Tandem Repeat; WGS = Whole Genome Sequencing