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Australasian College
for Infection Prevention and Control

ACIPC Toolkit

Air Sampling

ACIPC Toolkit: Air Sampling

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Acknowledgements

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Introduction

Health service organisations are required to have processes and procedures in place to minimise the infection risk to patients, older persons, consumers and the workforce from environmental hazards, including construction and maintenance activities, repair and upgrading of buildings, equipment, furniture and fittings[2].

The purpose of this toolkit is to provide guidance to the IPC requirements and considerations for microbiological air sampling activities within health services.

Background

Air flow is the mode of transportation of microbes from contaminated sources including soil, mouldy environments, dust and disturbances caused during construction and renovation activities[3]. Contaminated dust particles dispersed during construction activities may pose a health risk for patients, older persons, consumers and staff. Early planning of construction and renovation activity within the healthcare setting is required to reduce environmental hazards, to minimise environmental risks and implement controls to limit the spread of dust.

Outbreaks of infections associated with construction and renovation activities have been found to be related to healthcare facility plumbing and ventilation systems, including:

- Leaking water pipes that have damaged ceilings and contaminated intravenous supplies in a storeroom of items used to treat children with leukemia, who subsequently developed aspergillosis[4].

- A faulty ventilation system that circulated unfiltered air from an adjacent construction zone into patient care areas, resulting in an outbreak of aspergillosis[4].

People at risk

People at increased risk of healthcare associated infections (HAIs) related to construction and renovation activities include the immunosuppressed, those receiving chemotherapy or prolonged antibiotic and steroid therapies, organ transplant recipients, and immunodeficiency diseases[4]. Other at-risk cohorts include people receiving dialysis, mechanical ventilation, the very young and the very old[4].

Specialty units considered to be high risk include: Intensive Care Units (adult, paediatric and neonatal), Operating Theatres, Outpatients, Emergency Department, Radiology and Oncology wards[5].

Aerosols

Contaminants in the air (aerosols) include bacteria, fungi, viruses and pollens[6]. An aerosol is characterised as a particle, solid or liquid suspended in the air, with particle size ranging from $<1\mu m$

to $\geq 50\mu m$[6]. Particles can be single unattached organisms, or present in the form of clumps of a number of bacteria that also include dust and organic or inorganic materials[6].

Many factors influence the survivability of aerosolised microorganisms including temperature, humidity, exposure to UV or electromagnetic radiation, oxygen sensitivity and the suspension medium[6]. Some organisms can survive for long periods and can be carried considerable distances in the air while still remaining viable; these organisms may settle on surfaces and become airborne again as a secondary aerosol if activity occurs that disrupts them, e.g., sweeping or bed making[6].

Fungi and bacteria inhabit almost every building and are abundant and lay dormant in any place that gathers dust. When disturbed, microbes and fungal spores can be transported through the air and inhaled[5]. Construction-related HAIs have primarily been identified to be related to fungi, in particular *Aspergillus* species[4].

Aspergillus

Aspergillus is a spore-forming fungi that is widespread in nature and can be found in decaying organic matter, soil, compost and dust[7].

Aspergillosis is the term used to describe infections caused by *Aspergillus* species (spp.), with most cases of aspergillosis caused by the *Aspergillus fumigatus* species[8]. *Aspergillus spp.* infections are rare in humans, they most commonly occur in immunocompromised individuals and are associated with high rates of morbidity and mortality[8]. The infectious dose of *Aspergillus spp.* is unknown, and the incubation period for infection is estimated to be 2 days to 3 months[8]. *Aspergillus* spores are small and remain suspended in the air for prolonged periods, settling from the air slowly, which increases the risk of inhalation and contamination of environmental surfaces[4].

Environmental reservoirs for *aspergillus* include decomposing organic matter (soil and compost), contaminated damp materials including timber, plaster, potted indoor plants, air ducts contaminated with dust and bird excrement, and the outdoor environment[5,8]. *Aspergillus fumigatus* has been identified in dust and dirt that has accumulated within hospital ceilings[5]. Activities that disturb dust and dirt can disperse millions of spores throughout a facility, which can infect anyone who inhales it, particularly the immunocompromised[5]. Building and construction works within healthcare settings have been identified as a source of healthcare associated *Aspergillus spp.* infections[5].

Preventative strategies

Construction and renovation activities pose an increased risk for patients, older persons, healthcare workers and consumers, therefore a proactive approach must be taken to reduce the risk of HAIs. Preventative strategies include:

- Planning and risk assessments to identify and implement strategies to control the dispersion of dust during construction and renovation activities, and

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- Installation of building supply air systems designed and maintained to control the dispersion of fungal spores within occupied spaces through the use of high efficiency filters[5].

Other preventative strategies to control dust dispersion and reduce the risk to patients and older persons include:

- Moving immunocompromised patients and older persons away from the construction area.

- If construction is taking place adjacent to the healthcare facility, all windows, doors, intake and exhaust vents should be sealed to prevent air leaks into care areas. Risk assessments should be undertaken by the engineering team to assess the requirements for air intake pre-filters in high-risk settings.

- A sealed dust barrier (hoarding) should be created from floor to ceiling.

- If construction is taking place in a high-risk area, an anteroom must be in place for entry to the construction zone that is sealed and impermeable. Consideration for High Efficiency Particulate Air (HEPA) vacuuming of clothing prior to exiting the anteroom, if clothing sufficiently dusty by contractors.

- Air pressure within the construction zone should be negative compared to the adjacent areas.

- Air from within the construction zone should be exhausted outside or circulated through a HEPA filter.

- If the construction project involves air vents and spaces, they should be vacuumed with a HEPA filtered vacuum prior to construction commencing.

- Walk-off sticky mats (or similar alternative) should be placed outside the entrance to the construction zone and changed regularly when dust build up reduces efficacy.

- Areas adjacent to patient areas should be vacuumed daily and the frequency increased as needed, with a HEPA filtered vacuum.

- Construction supplies and debris should be in covered containers for transport to and from the construction zone.

- Consideration should be given to the route taken to transport construction supplies and debris from the construction zone, to minimise transit through patient care areas and timed to avoid high-traffic periods. Where lifts are used along the transit route, protective coverings should be implemented.

- Consideration of how construction debris is removed via trucks offsite with coverage or damping down so contaminated dust is not blown around by the trucks exiting the site

More information and sample audits of construction zones are available in the ACIPC Construction and Renovation Toolkit.

Operating theatre ventilation systems

The primary function of operating theatre ventilation systems is to prevent airborne microbes from entering a surgical wound. The links between operating theatre air quality and postoperative HAIs has been well established[9]. Specialised ventilation systems are required to be installed and maintained to provide high quality filtered air to operating rooms[9]. Specific requirements include directional airflow between rooms, air exchange rates and HEPA filtration.

Before a new operating theatre is used, or after a pre-existing operating theatre has undergone significant renovations that may impact the airflow pattern, commissioning of the space is required[9]. The commissioning process for the operating theatre includes[9]:

- Air distribution and flow within the operating theatre and between room assessments, e.g., from the operating theatre into the anaesthetic room and external corridor; from the anaesthetic room and scrub area into the operating theatre; and from adjacent rooms into the operating theatre.

- HEPA integrity testing should be undertaken to ensure penetration rates are within acceptable limits.

- The heating, ventilation and air conditioning (HVAC) unit should be confirmed to be functioning correctly by engineering experts.

- The air pressure and air changes per hour (AHC) rates should be checked and verified.

- Airborne microbial sampling results should fall within satisfactory parameters.

Air sampling

Microbiological air sampling is a tool used to determine the number and types of microorganisms or particulates in the air[6]. Air sampling in healthcare environments can be problematic and remains a contentious issue due to the lack of uniform air-quality standards. It is not recommended to be undertaken on a routine basis, though it can be considered as part of an Infection Prevention and Control (IPC) risk management program[5,6].

Air sampling will only measure air quality at a point in time and can be affected by multiple factors including indoor traffic, visitors and personnel entering the facility, temperature, humidity, time of day or year, effectiveness of cleaning of the area post works, and the performance of the HVAC system[5,6]. Other issues associated with microbiological air sampling include; a lack of protocols for testing, a lack of standards for linking fungal spores with infection rates with no identified safe level of exposure; variability of sampler readings and volumes of air sampled, and confounding variables with high risk patients including time spent outside without PPE[6].

Air sampling can be an expensive and time-consuming exercise that can be further complicated by multiple variables including the analysis and interpretation of results. As such, air sampling is recommended to be undertaken during the following circumstances:

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- To test the efficacy of HVAC systems and HEPA filters for commissioning and recommissioning of operating theatres, pharmacy preparation rooms and clean rooms.

- To identify background levels of air quality prior to and during construction and renovation activities.

- During investigations into clusters of infections where environmental reservoirs are suspected in disease transmission[5,6].

It is important to consider the time delay from air sampling to receiving results during the planning phase of air sampling activity. Results can take from 3–7 days to be finalised[10], which may result in delays in commissioning or operational activity. For example, operating theatres that require air sampling, should not be used until acceptable results have been returned. Results outside of acceptable parameters may require further cleaning and testing prior to usilisation[10].

Sample site selection

Comparison samples may be required to determine the baseline concentration of fungi in an area and determine the effectiveness of filtration and containment measures[11]. For example, during construction and renovation activities, baseline assessment of air quality prior to the commencement of construction activities that includes site clearance or demolition will provide a comparison reference point[11]. For this type of sampling, the level of fungi within the protected site may be compared to levels adjacent to the site that are not controlled and are outdoors[11].

Sample site selection must be tailored to the goals of undertaking air sampling; for example, in an outbreak of HAIs, the air sample results in the rooms of the affected might also be compared with the sample results in the rooms of the unaffected[11]. Isolates detected during cluster infection events may need molecular typing and comparison to patient isolates for confirmation[6].

Active air sampling

Active air sampling uses a machine that physically draws air through the air sampling device and over a sampling plate of culture media. The quantity of microorganisms present is measured in colony forming units per cubic meter of air (m^3)[5,12]. Active air sampling is the most widely used and preferred method of air sampling.

Passive air sampling

Passive air sampling uses settle plates, standard petri dishes containing a culture media, that are exposed to the air for a given time to collect particles that settle on them. Results are usually expressed as CFU/plate/time or as $CFUm^2$/hr[12] [11]. Passive air sampling provides little data that is considered useful due to the buoyant nature of airborne particles and the different rates they settle at. They are also considered to be insufficiently sensitive to detect fungi pathogens[11].

Particle counters

Particle counters count the number of airborne particles in the air, and can verify the effectiveness of dust control measures[11]. Particle counters do not measure microbial aerosols; however, they can

be used to measure air quality in occupational settings during construction and renovation activities to determine if barriers are effective at controlling dust dispersion, and when performed near the perimeter of the hoarding can identify gaps in joints or seals that require repair[6].

Particulate air sampling does not require laboratory services for reporting on results[6], and there is no acceptable threshold level, as the results are greatly impacted by foot traffic and activity in an area, therefore comparison samples are required to assess the effectiveness of control measures[11].

Performing air sampling

Prior to air sampling taking place, an assessment must be completed to determine the health service's ability to perform air sampling, including the training of the people undertaking air sampling, the laboratory service processing the samples, and the organisation's ability to interpret results. National Association of Testing Authority (NATA) accredited laboratories for environmental testing should be sourced to process testing samples. Consideration should be given to the use of an accredited service provider to provide a trained and experienced technician to undertake air sampling, process the samples, and provide interpretation of the results.

Air sampling should be performed according to the manufacturer's instructions of the equipment being used, and the air sampler should be checked, calibrated and serviced according to the manufacturer's instructions[6]. This information should be verified prior to use. Consideration should be given to time-delay air sampling machines, to reduce the risk of contamination by the operator.

Areas being sampled should be thoroughly cleaned prior to sampling taking place, including ceilings, walls, ventilation grills, all horizontal and vertical surfaces and fixed equipment. For operating theatre environments, a second clean prior to sampling is recommended to ensure all contamination is removed[10]. HVAC systems must have been running continuously for a minimum of 24 hours prior to air sampling, during this time all construction activity must have been ceased, however cleaning can take place[10].

In a clean, well-ventilated operating theatre, the main source of airborne contamination is contaminated skin particles from people. It is therefore imperative that air sampling takes place only when there are no people in the operating theatre or adjacent rooms (e.g., set up rooms) that can impact air flow and quality[9]. Air sampling should be performed in undisturbed conditions, as air sampling after human activity such as walking or vacuuming has taken place is likely to result in a higher number of airborne microorganisms detected[6]. Personnel undertaking the air sampling should be dressed in operating theatre attire including hair covering and surgical mask, and use aseptic technique practices[10]. The most appropriate time for air sampling to take place is before room use, for example early morning before operating theatre personnel arrive[9]. The space being sampled must be empty of all non-fixed items, including consumables, sterile stock and mobile equipment[10]

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## Air Sampling strategy for operating theatre suites

The number of sample collections sites can vary depending on the size of the space being sampled and the type of ventilation system in place. Air supplied to an operating theatre must be delivered in a way that minimises recirculation of air and provides the cleanest air over the operating table area[1]. There are two main operating theatre ventilation systems, conventional ventilated and ultraclean ventilation[1].

Conventional ventilated operating theatre (turbulent airflow)

In a conventional ventilated operating theatre, supply air is introduced from the ceiling, then moves downwards towards the floor and exits through vents located in the sides of the room, this design creates a continuous flow of clean air that dilutes contaminants and particles[1].

Air sampling in a conventional ventilated operating theatre should consist of at least two sample sites per room, to reduce the possibility of technical errors[9]. At a minimum, samples should be collected from the centre of each room (e.g., operating theatre, set-up room, sterile storeroom) in a position considered to simulate the exposure, for example in an operating theatre the air sampler should be placed in the centre of the operating table.

Figure 1: Conventional Theatre with terminal HEPA units
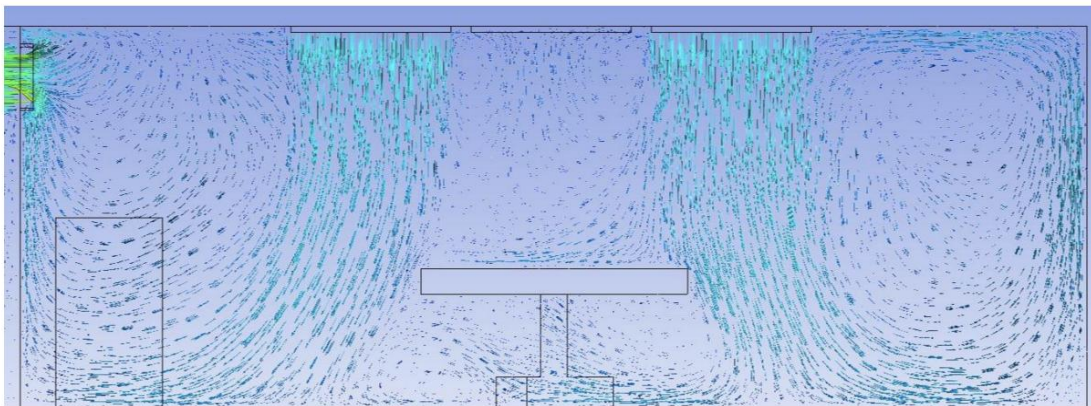
Source: Engineering guidelines for healthcare facilities: Volume 4 – Heating, ventilation and air conditioning[1]

Ultraclean ventilated (UCV) operating theatres (laminar airflow)

UVC ventilation provides a large volume of clean air to the air flow zone, in which the operating table and instrumentation is typically placed and exposed, and the airflow in and around the clean zone serves to prevent particles from outside the zone from entering[1].

Air sampling in an ultraclean theatre should sample each corner of the unidirectional air flow zone perimeter, halfway along each side of the perimeter, one in each corner of the inner zone and one in the centre of the zone[9].

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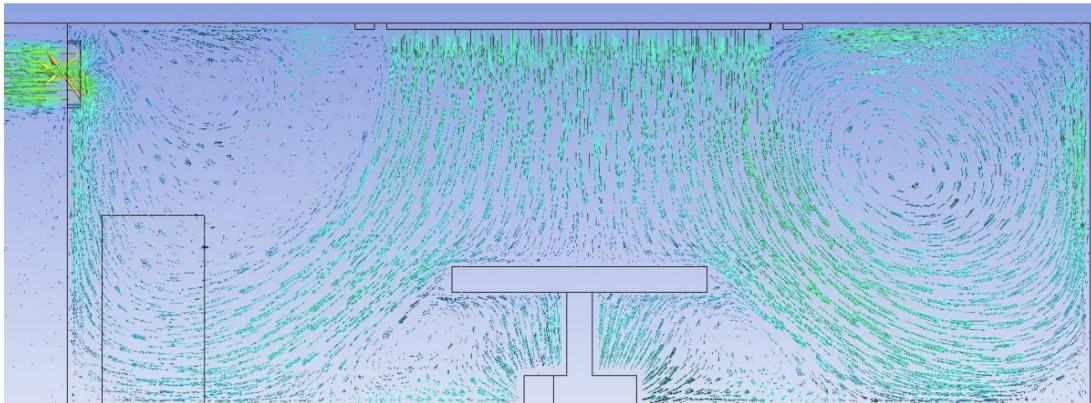P +61 3 6281 9239 | E office@acipc.org.au | W www.acipc.org.au

Figure 2: UCV operating theatre

Source: Engineering guidelines for healthcare facilities: Volume 4 – Heating, ventilation and air conditioning[1]

Air sampling reference diagram

When air sampling is completed a diagram of the room being sampled and the position of the sampling plates should be added, so there is a reference document to refer to when reviewing results.

Comparison air sample plate

Consideration should be given to a comparison reference sample plate that is taken outside of the area being tested to provide baseline comparison data. For example, in an operating theatre the comparison sample could be taken in the external corridor.

Air sampling reference diagram example
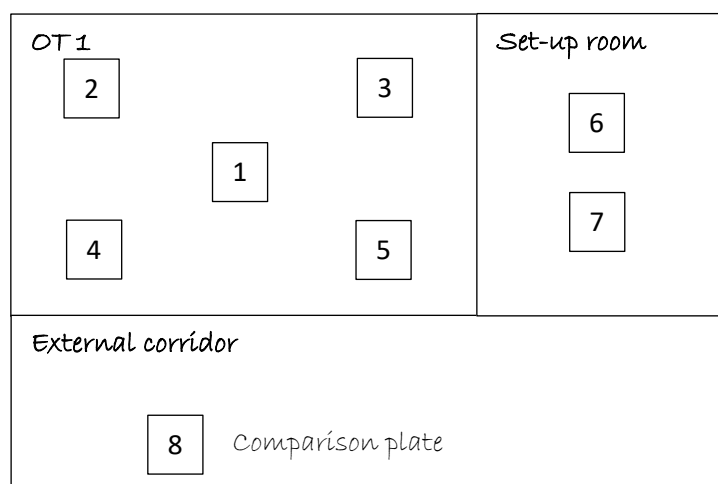
Air Sampling for cleanrooms and controlled environments

Cleanrooms and controlled environments are areas that have systems in place to control airborne particulate contamination to levels that are appropriate for sensitive activities, including industries involved in pharmaceuticals and medical devices[13].

Cleanroom monitoring includes the use of settle plates and active air sampling, as well as monitoring of temperature, humidity and air pressure. The PIC/S GMP Guide PE 009 *Guide to Good Manufacturing Practices for Medicinal Products,* provides requirements for environmental monitoring and interpretation of results for cleanrooms.

Air sampling in cleanroom environments should be undertaken based on the Australian Pharmacy regulations, and local health authority guidelines or recommendations.

Further information can be found at:

- Australasian Health Facility Guidelines, Part B – Health Facility Briefing and Planning 0560 – Pharmacy Unit https://aushfg-prod-com-au.s3.amazonaws.com/HPU_B.0560.0_7%203_0.pdf

- AS/ISO 14644-1: 2017 Classification of air cleanliness by particle concentration

- AS/ISO 14644-2: 2017 Monitoring to provide evidence of cleanroom performance relating to air cleanliness by particle concentration.

- Pharmaceutical Inspection Convention (PIC/S) Guide to good manufacturing practice for medicinal products, PE 009-16 https://www.tga.gov.au/resources/publication/publications/pics-guide-gmp-medicinal-products-version-16

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Results and data interpretation

Air sampling results are considered meaningful when compared to results taken from defined areas, time periods and conditions[6]. Fungi present in a controlled environment must be quantified and their numbers compared with those present in the air of an uncontrolled environment[11]. Acceptable test result parameters should be established prior to testing commencing and should be based on local health authority guidelines or recommendations, with consideration to historical results.

Preliminary results are usually not available until at least 48 hours after the samples are received by the laboratory, with final results on day 7; results received before that time may provide misleading information[10].

Results must be discussed with IPC personnel, a clinical microbiologist, construction Project lead, and relevant clinical personnel/representatives. Results that are outside of the acceptable range should be reviewed to establish if re-testing is required, in which the engineering and maintenance support personnel must be engaged to review the HVAC systems and confirm functionality prior to re-testing. Repeat environmental cleaning is recommended.

Air sampling results are contentious due to the lack of accepted standards. There are currently no guidelines for the interpretation of fungal counts within the general environment, however, fungal counts should not vary significantly from the baseline values[5].
Below is a summary table of recommended sampling results.

Table 1. Summary table of Australian recommended air sampling results.

	Environment	Bacterial counts	Aspergillus
Western Australia	Operating theatre	≤10 CFU/m^3	≤1 CFU/m^3
Victoria[5]	Operating theatre/ protective environment (12 ACH and positive pressure)	≤15 CFU/m^3	<0.1 CFU/m^3
	Area with at risk patients		<1 CFU/m^3
	General ward areas		<4 CFU/m^3
New South Wales[14]	Outdoor air		5–10 CFU/m^3
	HEPA filtered air, >99.95% efficiency and >10 ACH		<1 CFU/m^3
	In ward area, no filtration		<5 CFU/m^3
South Australia	Ultraclean operating theatre (HEPA filtered, laminar flow)	<0.5 CFU/m^3 or one bacterial colony for each 2 cubic metres air sampled	<0.5 CFU/m^3 or one fungal colony for each 2 cubic metres air sampled
	Conventionally ventilated operating theatre or procedure room (may be HEPA filtered, turbulent flow)	<10 CFU/m^3 bacteria or not exceeding historical counts if these are lower	<10 CFU/m^3 fungi or not exceeding historical counts if these are lower

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Table 2. Summary table of International recommended air sampling results.

	Environment	Bacterial counts	Aspergillus
Ireland[15]	Outdoor air		5–10 CFU/m^3
	HEPA filtered air, >99.9% efficiency & >10 ACH		<1 CFU/m^3
	In ward area, no filtration		<5 CFU/m^3
Scotland[16]	Operating theatre	<10 CFU/m^3	<10 CFU/m^3
United Kingdom	Ultraclean operating theatre (HEPA filtered, laminar flow)	<0.5 CFU/m^3 or one bacterial colony for each 2 cubic metres air sampled	<0.5 CFU/m^3 or one fungal colony for each 2 cubic metres air sampled
	Conventionally ventilated operating theatre or procedure room (may be HEPA filtered, turbulent flow)	<10 CFU/m^3 bacteria or not exceeding historical counts if these are lower	<10 CFU/m^3 fungi or not exceeding historical counts if these are lower
United States (CDC)	HEPA $\geq$ 12 ACH and 99.97% efficiency		<0.1 CFU/ m^3

Abnormal results and corrective actions

If high bacterial or *Aspergillus* counts are recorded, or healthcare associated aspergillosis is suspected, further investigation and a risk assessment is required including[15]:

- Review of the sampling process, including aseptic technique practice.
- Review of the engineering systems and parameters to ensure functionality and performance
  - Filters – clean and functional, correctly fitted
  - Positive pressure and air flow direction
  - Air exchange rate and air volumes
- Assessment of the following during periods of sampling:
  - Dust
  - Fabrics – present in the sampling zone
  - Ventilation ducts – clean and operational
  - Fans – clean
  - Ceiling voids – sealed, cleaned
  - Kitchen areas
  - Outside environmental sources

The area should undergo repeat terminal cleaning prior to further testing.

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Definitions

Active air sampling	An air sampling device draws air though the machine directly onto the surface of the medium
Air changes per hour (ACH)	Air changes per hour – the ratio of the airflow rate to the room volume; the ratio is expressed as the number of air changes per hour[6].
Air sampler	A device that draws a sample of air onto a medium to evaluate air quality.
CFU/m³	Colony forming units per cubic meter of air
Clean air	Air filtered through high-efficacy filters (90–95% filtration)
Cleanest air	HEPA filtered air
Clean room	A room in which airborne particle concentration is controlled, and constructed in a way to minimise the introduction, generation and retention of particles in the room[13].
Dirty air	Outdoor air
Dust	The air suspension of particles of solid material
FF&E	Furniture, Fixtures and Equipment
HEPA filter	High Efficiency Particulate Air filter that can remove 99.97% of particles $0.3\mu m$ in diameter[6].
HVAC	Heating, Ventilation and Air Conditioning systems
Healthcare facility	The building and facilities in which care is provided, including visits, short stay or permanent.
Healthcare setting	Places and services where healthcare occurs, including acute care hospitals, urgent care centres, rehabilitation centres, aged and disability residential care, specialised outpatient services (e.g., haemodialysis, dentistry, and office-based services), and community care.
Healthcare worker	Anyone who works in a healthcare or social care setting, e.g., medical practitioners, nurses, midwives, carers, dentists, allied health, students on placement, as well as executives, managers and administration personnel.
High-efficiency filter	A filter with particle-removal efficiency of 90–95%
IPC	Infection Prevention and Control
IPCRA	Infection Prevention and Control Risk Assessment
Laminar flow	HEPA-filtered air that is directed into a room in a unidirectional pattern[6].
Older person	An older person refers to an individual who has reached a later stage in life, typically 65 years or older. This stage is marked by physical and sometimes cognitive changes.
Operating theatre	A room where surgical or invasive procedures are performed
Outdoor air	Air taken from the external environment and not circulated through a ventilation system[6].
PPE	Personal Protective Equipment

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Passive air-sampling	Air sampling via sedimentation. When microorganisms settle on a surface over a period of time.
Positive pressure	The air pressure differential between two adjacent air spaces; whereby the pressure in the identified area is greater than that of the adjacent area/s.
Settle plates	A petri dish containing culture media, that is exposed to the air during passive air sampling.

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Version

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1.0	August 2024	New document	IPC Consultant	PGC
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Appendix 1: Air sampling reference tool

Site:		Date:
Area being sampled:		Time:
Name & position:		
Reason for sampling:		

Pre-sampling checklist

Item	Yes	No	NA
Terminal clean completed (2x for OT environment)			
HVAC system running >24 hours			
Access to room has been restricted			
Room is free from consumables, movable items			
Number of sites to be sampled determined			

Air Sampling Reference Diagram

Sample site

Sample number	Location	Volume of air sampled (Liters)	Results 48hrs	Results 7 days

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