Hazardous biological agents and the occupational exposure risks thereof in an animal testing facility

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First, I would like to thank my Heavenly Father for walking this path with me, granting me the opportunity, strength and knowledge to complete this major task.

Psalm 23: 4

Selfs al gaan ek deur donker dieptes,
sal ek nie bang wees nie,
want U is by my.
In U hande is ek veilig.

My parents, for loving, supporting and encouraging me in more ways than I can count. I cannot express the gratitude that I feel. Thank you for providing a safe space where I can always return to when life gets too overwhelming.

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PREFACE

This dissertation is presented for examination to fulfil the requirements of the Master of Health Sciences in the Occupational Hygiene degree at the North-West University. This dissertation is written in accordance with the NWU Harvard Style and United Kingdom English spelling, with exceptions made for the names and references used.

Chapter 1 includes a brief introduction to hazardous biological agents, their legislative guidelines and the role they play in posing a health risk to workers in an animal testing facility. This section also contains the problem statement, research aims and objectives, as well as the research question.

Chapter 2 consists of a detailed literature review regarding the relevant topics to this study, including the history of hazardous biological agents and the industries they are most prominent in, an in-depth review of animal testing facilities including the general facility design, animal bedding and personal protective equipment requirements. The chapter also includes a section on laboratory accidents and a few examples throughout history and their outcomes. Lastly, it contains methodologies on bioaerosol sampling, measuring physical parameters and genomic analysis.

Chapter 3 is written as an article entitled: “Hazardous biological agents and the occupational exposure risks thereof in an animal testing facility”, written following the NWU Harvard Referencing Guide.

Chapter 4 provides a conclusion, recommendations for possible control measures, as well as recommendations for future studies.

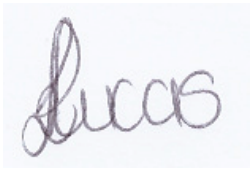
AUTHOR CONTRIBUTIONS

This study was planned and completed by a group of people. The contribution of each is listed in the table below:

Table A.1.1: Contributions of team members

Name	**Contribution**
Miss L Lucas (Student)	• Designed and planned the study. • Literature searches. • Data collection. • Statistical analysis and interpretation of data. • Wrote the dissertation.
Prof. SR Horn (Supervisor)	• Assisted with approval of the protocol and obtained ethics approval, interpretation of results, and documentation of the study. • Provided guidance on scientific aspects of the study. • Assisted with the planning and designing of the study. • Provided professional input and recommendations. • Reviewed the dissertation.
Prof. A Franken (Co-supervisor)	• Assisted with approval of the protocol, interpretation of results, and documentation of the study. • Provided guidance on scientific aspects of the study. • Assisted with the planning and designing of the study. • Provided professional input and recommendations. • Reviewed the dissertation.

I declare that I have approved this dissertation and that my role in the study, as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of L. Lucas's MHSc in Occupational Hygiene dissertation.







Miss L Lucas	Prof SR Horn	Prof A Franken

EXECUTIVE SUMMARY

Introduction

A hazardous biological agent (HBA) may cause negative health effects to any person who may be exposed to it. HBAs are in abundance everywhere around us at all times, including the workplace. The workplace of most concern is animal testing facilities due to the handling of animals and the possible exposure to contaminated animal bedding, food particles, and water droplets found in the facility's environment. Employees working in these facilities face a unique exposure scenario to HBAs. However, despite this fact, not many studies have been published regarding the HBAs that can be found in animal testing facilities and the potential health implications they may have on their employees.

Aims and objectives

This study aimed to determine the presence of hazardous biological agents within an animal testing facility and identify these agents. The first aim of the study was to collect air samples using active and passive microbial sampling in selected rooms within the facility. The second objective was to conduct genomic analysis using the samples collected from the first objective. The third objective was to measure the physical parameters such as temperature, relative humidity, airflow, carbon monoxide and carbon dioxide concentrations and correlate these values to the presence of microbial growth in the facility. Lastly, the fourth objective was to conduct real-time monitoring of dust particulate matter to assess their presence and correlate the concentration of dust with the bioaerosol growth in each area.

Method

Bioaerosol (airborne microorganism) samples were collected using an active microbial air sampler (MAS-100 NT®) to capture culture-dependent microorganisms as well as a TE-BC251 bioaerosol cyclone to capture size-selective culture-independent microorganisms. Passive sampling was done via surface swabbing and contact plates to collect any microbes that had settled onto surfaces. Settle plates were also used to capture any settling particles from the air that may contaminate surfaces. Real-time dust particulate monitoring took place using the Nanozen DustCount 9000 to assess the average concentrations of dust present in each area as well as the abundance of size fractions below 3 µm. The physical parameters, such as temperature, relative humidity, airflow, carbon monoxide and carbon dioxide concentrations, were also measured using handheld devices (TSI IAQ-Calc™ Indoor Air Quality Meter 7545 and TSI Airflow™ TA465-P Air Velocity Meter). Bioaerosol samples collected through culture-dependent and culture-independent methods were processed for downstream metabarcoding analysis. The biological samples underwent metabarcoding using nanopore sequencing via the Oxford Nanopore Technologies MinION device.

Results

The cloak room provided the highest concentration of microbial growth of all the rooms sampled in the facility. The microbial air sampler yielded the highest concentrations of microbial growth of all the sampling methods used. Genomic analysis of the colonies enabled the identification of the microorganisms to the genus and species level, which were classified into the different risk groups prescribed by the Hazardous Biological Agents Regulations, 2022 of South Africa. A total of 59 risk group 2 agents, of which 53 were bacterial and six were fungal, were identified. Furthermore, three risk group 3 agents, *Shigella dysenteriae*, *Escherichia coli*, and members of the *Bacillus cereus* group (which includes *Bacillus anthracis*), were detected in the facility. Due to the limitations of the nanopore sequencing method used, additional sample analysis using gold standard molecular techniques should be done to confirm the identity of *B. anthracis* with greater certainty. No statistically significant correlations were found between the physical parameters and the concentrations of microbial growth or the dust particulate matter and microbial growth.

The cloak room had the highest average relative humidity and carbon dioxide concentration (37.5% and 777 ppm, respectively), and the dirty hallway had the highest temperature and carbon monoxide concentration (21°C and 0.4 ppm, respectively) of all the rooms sampled in the facility. All air velocity measurements were below 0.1 m/s, and the average dust particulate matter concentration per room was below 0.001 mg/m^3. When assessing the dust particulate size fractions, there was a higher concentration of smaller (< 1 µm) dust particles found compared to larger particles (2–3 µm).

Conclusion

The low concentrations of microbial growth found in the animal testing facility reduce the likelihood of the workers being exposed to HBAs. However, the 59 risk group 2 agents and the three risk group 3 agents identified pose a serious health hazard to those workers who might be exposed. The combination of small dust particulates in the air and low air velocity could indicate a problem with the ventilation system. It indicates that the larger dust particles settle quicker onto the various surfaces of the facility and the smaller dust particles stay airborne for longer periods of time, increasing the likelihood of a worker inhaling these particles. This fact increases the risk to health as dust particles can house HBAs and transport them to the gas exchange regions of the lungs and cause serious infections that can easily spread throughout the body. When assessing all the exposure factors discussed, such as the severity of consequences, likelihood of exposure, and possibility to cause harm, as well as the effectiveness of the current control measures, the conclusion can be made that the HBAs found in this animal testing facility pose a moderate risk to its workers' health.

Key terms: Oxford nanopore sequencing, HBA risk groups, bioaerosols, biosafety level laboratory, genomic analysis

LIST OF SYMBOLS AND ABBREVIATIONS

Symbols

%	Percentage
<	Less than
=	Equal to
>	Greater than
±	Plus-minus
≤	Less than or equal to
≥	Greater than or equal to
°	Degrees
°C	Degrees Celsius
µl	Microlitre
1000 counts/m^3	Thousand counts per cubic metre
CFU	Colony forming unit
CFU/cm^2	Colony forming units per square centimetres
CFU/cm^2/h	Colony forming units per square centimetres per hour
CFU/g	Colony forming units per gram
CFU/m^3	Colony forming units per cubic metre
CFU/plate	Colony forming units per plate
cm	Centimetre
cm^2	Square centimetre
g	Gram
L/min	Litres per minute
m	Metre
m/s	Metre per second
m^2	Square metre
m^3	Cubic metre
mg/m^3	Milligrams per cubic metre
ml	Millilitre
mm	Millimetre
ng	Nanogram
ng/µl	Nanogram per microlitre
ng/g	Nanogram per gram
nm	Nanometre
ppm	Parts per million
µm	Micrometre

Abbreviations

AC vent	Air supply ventilation
BSL	Biosafety level
CDC	Centers for Disease Control and Prevention
CO	Carbon monoxide
CO_2	Carbon dioxide
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDC	Electrostatic dust cloths
EX vent	Extraction ventilation
GAS	Group A Streptococcus
GSP	Gesamtstaubprobenahme
HBA	Hazardous biological agent
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEPA	High-efficiency particulate air
HIV	Human immunodeficiency virus
HPV	Human Papillomavirus
HREC	Health Research Ethics Committee
HVAC	Heating, ventilation, and air conditioning
IAQ	Indoor air quality
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
ITS	Internal Transcribed Spacer
LCM	Lymphocytic choriomeningitis
MAS	Microbial air sampler
MCE	Mixed cellulose esters
NA	Nutrient agar
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NWU	North-West University
OEL	Occupational exposure limit
OHHRI	Occupational Hygiene and Health Research Initiative
ONT	Oxford Nanopore Technologies
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PM	Particulate matter

PPE	Personal protective equipment
RH	Relative humidity
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SARS	Severe acute respiratory syndrome
SOP	Standard operating procedure
SPF	Specific pathogen-free
TSA	Tryptic soy agar
UCLA	University of California, Los Angeles
UVGI	Ultraviolet germicidal irradiation
WHO	World Health Organisation

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CHAPTER 1 INTRODUCTION

1.1 Background

1.1.1 Introduction to hazardous biological agents

A hazardous biological agent (HBA) is defined as an “agent which may cause infection, allergy or toxicity or otherwise create a risk to human health” (Department of Employment and Labour, 2022:4). The term pathogen can also be used for a microorganism that causes a disease to humans. There are many different types of pathogens, such as bacteria, fungi, viruses, archaea and allergens such as pollen and endotoxins (Ehrlich, 2016:177).

Bioaerosols are airborne particulates comprised of complex mixtures of microorganisms, living or dead organisms, fragments of cells and biomolecules. The size of microorganisms in bioaerosols varies significantly, with viruses being the smallest (10 nm to 0.2 µm), followed by bacteria (0.3 to 10 µm), and then larger structures like fungal spores (0.5 to 30 µm) and pollen (10 to 100 µm). This wide size range means they can travel through the air and reach different parts of the respiratory system, depending on their size (Li *et al*., 2023:463; Whitby *et al*., 2022:104−105). Bioaerosols can be generated from both indoor and outdoor environments from various anthropogenic and natural sources. Examples of indoor sources include animals, organic waste, plants and humans (talking, sneezing and coughing) as well as outdoor air contaminants from farms, composting plants and waste treatment plants that can enter the indoor environment (Cox *et al.*, 2019:572−573). It is, however, difficult to determine the health effects of bioaerosols as various factors, such as their abundance, composition, distribution and dose-response relationship, still lack understanding. There is also a lack of information on how other air pollutants, such as dust particulate matter and chemical agents (Li *et al*., 2023:463), interact with bioaerosols to human health effects, and this makes the exposure to bioaerosols difficult to quantify (Whitby *et al*., 2022:104−105). The integrity and effectiveness of the body’s defence mechanisms can also influence the possibility of health effects when exposed to HBAs (Kourilsky, 2016:1).

The body relies on several lines of defence to protect against exposure to pathogens, each aligned with the major routes of exposure. The first line of defence consists of physical and chemical barriers that prevent pathogens from gaining entry. Within the respiratory system, mucous membranes, nasal hairs and the tonsillar ring intercept inhaled microorganisms, while coughing and sneezing expel those that bypass these initial barriers (Ehrlich, 2016:171). These mechanisms are particularly important because bioaerosols, due to their small size, can penetrate deeply into the lungs, reaching the oxygen exchange regions (Schröder, 2014:244). The skin also serves as a crucial barrier; its acidic mantle creates conditions that are inhospitable to most bacteria. Ingestion-related exposures are

counteracted by the digestive system, where acids and enzymes destroy many pathogens (Ehrlich, 2016:171).

When these external barriers are breached, for example through cuts, abrasions or lesions in the skin, pathogens can enter the body directly. Small airborne particles may also bypass the respiratory system's first line of defence and reach the deeper lung regions. In such cases, the body must rely on its second line of defence, which includes the lymphatic system and specialised white blood cells that identify, attack and eliminate invading micro-organisms. Their actions also contribute to the production of antibodies, thereby enhancing protection against future infections (Ehrlich, 2016:171).

1.1.2 Animal testing facilities

Animal testing facilities are research facilities or laboratories with the specific purpose of conducting research using animals. Although biomedical research has advanced so far that the need for animal test subjects is reduced, it remains an integral part of scientific discovery and medical advancement (Chang & Grieder, 2024:295). Many different programs utilise animal testing facilities, such as research organisations serving industries (e.g. pharmaceutical industry), colleges and universities, government-funded research institutes, as well as private nonprofit research institutes. These facilities' designs, size and resources can vary due to determining factors such as the type of research being performed, the type of animals being housed and the size of the research enterprise that the facility forms part of. There are a wide variety of animals that can be utilised in these facilities, such as rabbits, dogs, pigs, goats, sheep, reptiles, fish, and amphibians, however, rodents are most commonly used due to their genetic similarity with humans (Leszczynski, 2018:599–638; University of Basel, 2025). The exposure scenario of workers to HBAs in an animal testing facility is of great concern, leading to its classification as a high-exposure occupation. This is due to the risk that the workers may be exposed to urinary protein allergens, faecal matter, as well as zoonotic microorganisms while handling the animals or their bedding, food and/or water (Van den Heever, 2014:199–200). Exposure may occur via the following scenarios: inhalation of micro-organisms or particulate matter (PM) carrying micro-organisms, absorption through damaged or weak areas of the skin, accidental needle stick injuries or animal bites/scratches as well as the ingestion of contaminated water droplets or food due to the improper use of in place control measures (Davidson *et al.*, 2020:490–491).

1.1.3 Regulations and standards

The South African Hazardous Biological Agents Regulations of 2022 do not contain any limits to which exposure levels could be compared to like the South African Hazardous Chemical Agents Regulations of 2021 with their occupational exposure limits (OEL) (Department of Employment and Labour, 2021:8; Department of Employment and Labour, 2022:24). This complicates the interpretation of results obtained, as exposure to HBAs cannot be evaluated in the same manner as hazardous chemical agents. This is due to the unique immunological responses of individuals and the nature of HBAs. The likelihood of infection is dependent on the exposure and the strength of the receiving individual's immune system. The type of agent is also more important than the dosage of the agent, because when a person is exposed to an agent, no matter how minimal, they can get infected (Davidson *et al.,* 2020:507). However, the South African Hazardous Biological Agents Regulations of 2022 contain a risk group classification, where an agent is divided into one of four risk groups, depending on the probability to cause human disease, to infect the community and if adequate prophylaxis and treatment are available (Department of Employment and Labour, 2022:23). The groups are as follows:

- **Risk group 1**: Unlikely to cause human disease.
- **Risk group 2**: May be hazardous to exposed persons and cause human disease; however, it is unlikely to spread to the community. Effective prophylaxis and treatments are generally available.
- **Risk group 3**: Presents as a serious hazard to exposed persons and may cause severe human disease; it also presents a risk of spreading to the community. Effective prophylaxis and treatments are available.
- **Risk group 4**: A serious hazard to exposed persons and causes severe human disease; it also presents a high risk of spreading to the community. No effective prophylaxis and treatments are available.

Regulations 6 and 7 of the Hazardous Biological Agent Regulations of 2022 in South Africa (Department of Employment and Labour, 2022:9–11) require that a workplace must conduct biological risk assessments and exposure monitoring, at intervals not exceeding 24 months, if there is a risk that a person may be exposed to HBAs in the workplace. This means that there should be a record of the type of HBAs, the effects of exposure and the probability of exposure in animal testing facilities. However, there is a lack of public information regarding the risks inside an animal testing facility due to the monitoring reports remaining confidential within the facility. This leads to the need for more information regarding the HBAs that can be found in animal testing facilities to be published.

The system of biosafety level (BSL) classification was implemented globally to help mitigate the risk of accidental infection. The classification consists of four levels and is dependent on the type of agents that are used in the laboratory. The specific classification then provides guidelines that should be followed to ensure the safety of the workers within the facility (Meechan & Potts, 2020:71–117). The levels, type of agents worked with, safety guidelines and agent examples are listed in Table 1.1.

Table 1.1: Biosafety level (BSL) classification (Princeton University, 2025).

BSL	Agents	Safety practices	Example
BSL-1	Biological agents that are not known to cause human disease in immunocompetent adult humans	Basic levels of protection for specific biological agents such as PPE, lab coats and disposable gloves.	Adeno-associated virus
BSL-2	Agents that pose a moderate risk of human disease and harm to the environment	Limit access and include occupational medical surveillance, including use of PPE such as lab coats and disposable gloves	*Staphylococcus aureus*
BSL-3	Indigenous or exotic agents with known potential for aerosol transmission	Viable material moved in primary and secondary containers and only opened in a BSL-3 laboratory. Standard PPE and solid front gowns, two pairs of gloves, scrubs or coveralls is required	*Mycobacterium tuberculosis* bacterium
BSL-4	Dangerous and exotic agents that pose a high risk of fatal diseases	Change clothes before entry and shower upon exit, and daily inspections of essential containment and life support systems are required, use of standard and special PPE such as solid front gowns, gloves, coveralls, positive pressure-, full-body-, air supply or positive pressure suits is required	Ebola virus

PPE = personal protective equipment

The sampled facility in this study is a BSL-2 facility and makes use of basic PPE such as lab coats and surgical gloves with the inclusion of other PPE, such as surgical masks, hair bonnets and shoe covers, to protect the integrity of the research being conducted. Lipman and Leary (2015:1588–1589) states that, even though the facility provides basic protection for their workers, the safety measures found in an animal testing facility are more protective of the animals and research than the workers.

1.2 Problem statement

Animal testing facilities present a unique environment with multiple potential exposure pathways which workers could be exposed to HBAs. The safety measures found in animal testing facilities are more protective towards the animals and the research than of the workers. There is a risk that the worker may inhale urinary proteins and faecal matter from the bedding, inhale aerosolised animal food particles and water droplets, and experience bites and scratches from the animals. There are many possible areas where accidental cross contamination can occur from the animals to the workers. This means that the HBAs found in an animal testing facility can be harmful to human health if exposure is not correctly controlled.

1.3 Research aims and objectives

The aim of this study was to determine the presence of hazardous biological agents within an animal testing facility, and furthermore to identify these agents.

The specific objectives of this study were to:

Objective 1:

Perform passive surface sampling and active air sampling within selected areas of the animal testing facility, to determine the presence of potentially hazardous biological agents.

Objective 2:

Identify the hazardous biological agents collected in objective 1 by use of genomic analysis and to categorise the HBAs into their respective risk groups according to the South African Regulations for Hazardous Biological Agents, 2022.

Objective 3:

Measure physical parameters such as airflow velocity, carbon monoxide and carbon dioxide concentration, temperature and relative humidity and furthermore to correlate the values with the concentrations of HBAs found, since these factors may influence the presence of hazardous biological agents in the animal testing facility.

Objective 4:

Determine the presence of dust particulate matter by real-time sampling and to correlate its presence in relation with the concentration of HBAs found, since particulate matter acts as carriers of HBAs.

1.4 Research question

To what extent are the HBAs found in the animal testing facility hazardous to the workers' health, as categorised into the risk groups according to the South African Hazardous Biological Agents Regulations, 2022?

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CHAPTER 2 LITERATURE REVIEW

2.1 History of hazardous biological agents

Hazardous biological agents (HBAs) have played a significant role in the world's history, manifesting in the form of epidemics and pandemics. One of the most prominent is that of cholera, with the first of many recorded outbreaks beginning in 1817 and subsequently spreading across continents through repeated pandemic waves (Piret & Boivin, 2021:1). These outbreaks illustrate how highly transmissible pathogens can rapidly move through populations, especially in areas with poor sanitation and limited public health infrastructure. The most recent cholera outbreak is presented as a global epidemic affecting 26 countries worldwide as of 17 June 2025, demonstrating the continued vulnerability of modern societies to re-emerging infectious diseases (WHO, 2025). The World Health Organisation (WHO) has classified this epidemic (within a specific geographical area) as a grade three emergency with a global risk classification of very high, highlighting the scale, severity and international concern associated with the current event.

Considering both the ongoing global cholera epidemic and the latest global pandemic, Covid-19 (SARS-CoV-2), it is evident that HBAs pose a significant health risk to people worldwide, including those in workplaces. Covid-19 not only demonstrated how rapidly a novel pathogen can spread across multiple countries but also exposed shortcomings in biosafety preparedness, occupational health systems and institutional response capacities. As a result, the pandemic accelerated improvements related to HBA safety and control, particularly the strengthening of national regulatory frameworks. In South Africa, one of the most notable developments was the revision and updating of the Hazardous Biological Agents Regulations of 2001, now promulgated as the South African Hazardous Biological Agents Regulations of 2022. These updated regulations introduced clearer classifications, expanded employer obligations and reinforced preventive and reporting requirements, reflecting lessons learnt during Covid-19 and a renewed emphasis on protecting workers from HBAs (Department of Employment and Labour, 2022:22).

2.2 Hazardous biological agents in industry

HBAs are present in all workplaces, due to the nature and sources of HBAs. There are however workplaces where the exposure to HBAs is more prominent, especially those that work in close contact with animals, plant life and humans.

The main and most well-known industry of concern is that of healthcare and associated laboratory workers, due to the daily work with patients, blood and body fluids. This potential exposure scenario makes them highly susceptible to tuberculosis, influenza, the hepatitis B virus (HBV) and the human

immunodeficiency virus (HIV). Salawu and Farag (2024:1) outlines statistics, provided by the WHO, that each year, more than three million healthcare workers are exposed to bloodborne pathogens. Among these, approximately 2 million are exposed to HBV, 0.9 million to the hepatitis C virus (HCV), and 170 000 to HIV. The laboratory personnel also face occupational hazards, particularly at the clinical microbiological laboratories that work with infectious agents. Salawu and Farag (2024:1) states that the risk of transmission can be reduced by following regulatory and professional groups' safety rules such as, early detection and isolation of infectious agents, proper hand hygiene, worker training, adequate administrative controls, use of appropriate personal protective equipment and safer work practices.

Additional international literature supports these concerns, indicating that exposures to bloodborne pathogens remain common despite improvements in safety standards. A recent global review reported that high rates of occupational exposure still occur across high-, middle- and low-income healthcare systems, largely due to inconsistent training, insufficient engineering controls and under-reporting of incidents (Almutairi *et al*., 2025:54). Multi-year evidence from European hospitals similarly found that nurses, laboratory staff and cleaning personnel experienced repeated exposure to blood and body fluids over a five-year period, identifying these groups as particularly vulnerable due to their frequent handling of contaminated materials (Różańska *et al*., 2014:747). These findings highlight that, despite the availability of universal precautions, healthcare and laboratory workers remain at risk of exposure to HBAs, emphasising the need for strengthened safety cultures and more robust reporting and monitoring systems.

Furthermore, a veterinarian is a medical professional who works with animals and is also at risk of exposure to HBAs in the workplace. A questionnaire was sent out to veterinarians working in western Canada to evaluate the health effects of exposure to HBAs and HBA sources in the workplace (Epp & Waldner, 2012:144). The study found that only 16.7% of participants reported the occurrence of a zoonosis or exposure to rabies in a five-year timeframe where the most commonly reported event was ringworm. Eighty six percent of all bites and scratches were reported to be caused by cats, as well as the main cause of infection (81% of infections) was caused by cat bites and scratches. Another notable factor is the veterinarians who developed allergies during their careers. Approximately 38% of participants developed allergies, and 41% of them altered the way they practice due to their developed allergies.

Additional evidence reflects similar concerns globally. Veterinary fieldworkers in South Africa were found to be routinely exposed to zoonotic agents, with inconsistent PPE use and variable infection-control training contributing to the persistence of these risks (Mhlongo *et al*., 2025:1). International reviews confirm that veterinarians face a wide range of biological hazards, particularly during animal restraint, diagnostic procedures and necropsy, where bioaerosols and contaminated droplets may be

generated (Bonini *et al*., 2016:1). Collectively, the literature indicates that workers in the veterinary sector remain vulnerable to zoonotic HBAs across both clinical and field environments because of the unpredictability of animal disease patterns and the nature of daily work activities.

The agriculture industry is also an industry where exposure to HBAs is a prominent occupational risk. Mołocznik (2004:85) conducted a study in Poland to identify the agricultural activities where workers are exposed to HBAs, as well as the amount of time the workers spend conducting those activities. The study was conducted on 30 farms with production profiles of plants, animals and mixed profiles. Nineteen work activities were identified where the workers are exposed to HBAs; the most prominent source activities include spreading manure, plant harvesting and animal breeding. Plant production workers spent an average of 51% of their working time under HBA exposure conditions, compared to 80% for animal breeders and 77% for workers involved in mixed production. This is worrisome as most of these workers work extended hours, increasing their risk of exposure to HBAs.

More recent evidence reinforces these risks. A systematic review found that intensive livestock production commonly generates high concentrations of bioaerosols containing bacteria, fungi and endotoxins, posing respiratory and immunological risks to workers and nearby communities (Douglas *et al*., 2018:134). Workers in crop-farming environments also experience increased exposure to airborne dusts, spores and pesticide-contaminated aerosols, with studies showing that such exposures contribute to elevated respiratory symptoms compared to the general population (Mokarami *et al*., 2022:187). These findings highlight that agricultural workers are exposed to a wide range of airborne HBAs due to microbial proliferation in farming environments and the lack of engineered protective controls.

Medical research is an occupation that carries a risk of exposure to HBAs, with laboratory animal care workers being of particular concern, as they are classified as a high-exposure group. This classification is based on the possible exposure to urinary protein allergen inhalation and zoonotic micro-organisms (Van den Heever, 2014:213–214). Parthiban *et al.* (2021:563–564) noted that although animal experimentation is an integral part of research, the use of infectious agents on live animals increases the hazards associated exponentially. They state that there has been a surge of infectious diseases that have been transmitted from animals to humans recorded in recent years, indicating that this type of research poses a direct hazard to the personnel who work with the animals. Rim and Lim (2014:44–46) analysed the possible exposure scenarios and listed the diseases that the laboratory animal workers may contract. The exposure scenarios included handling the animals, specifying inhalation and percutaneous exposure, bites and scratches, as well as handling infected animal carcasses or placental tissue. The potential diseases that may be contracted include hantavirus infection, lymphocytic choriomeningitis (LCM), rat bite fever, Brucellosis, leptospirosis, influenza, Q fever, Hendra and Nipah virus diseases (Rim & Lim, 2014:44–46).

These concerns are echoed in broader laboratory-based research. A global scoping review found that laboratory-acquired infections and accidental pathogen escapes continue to occur worldwide, involving a wide range of bacterial, viral and fungal agents and arising primarily from procedural errors, aerosol-generating techniques and equipment failures (Blacksell *et al*., 2025:194). Similarly, a 50-year systematic review identified that laboratory-acquired infections occurred across laboratories operating at different biosafety levels, reinforcing that human error and procedural lapses remain key contributors to exposure, even in well-regulated environments (Zavaleta-Monestel *et al*., 2025:1).

2.3 Animal testing facility

2.3.1 Facility design

Animal testing facilities are laboratories that conduct tests on animals. Mice and rats are the most commonly used animals in these facilities, and they may also be genetically modified depending on the type of research being conducted. Rodents, like mice and rats, share substantial genetic similarities with humans, and their larger size makes them particularly valuable in studies that require greater volumes of sample material to obtain reliable results. They are also frequently used in behavioural research, as their learning abilities demonstrate a strong resemblance to those of humans (University of Basel, 2025). These facilities are typically designed with dedicated breeding rooms, allowing animals to be bred in-house to minimise the risk of introducing contaminated animals (Lipman & Leary, 2015:1576). Several types of environmental factors may influence the health of the animals, which can then, in turn, influence the experimental data obtained. Some of these variables include humidity, noise, population per cage, bedding, air and animal food (Huerkamp *et al.,* 2018). The facilities are designed in such a manner that they have control over these variables, which is also known as specific pathogen free (SPF) conditions. There is no natural ventilation or light used within these facilities due to the need to control these variables. This means that they make use of artificial light sources and mechanical ventilation systems, such as heating, ventilation, and air conditioning (HVAC) systems (Huerkamp *et al.,* 2018).

The animals are generally housed in micro-isolator caging systems with mass air displacement racks that contain high-efficiency particulate air (HEPA) filters and flexible-film isolators (Lipman & Leary, 2015:1548). This is done to serve as a barrier between the animals and the surrounding area to ensure the maintenance of the SPF animals. This means that the air that is circulated within the cages is separate from the air that is circulated within the room. This serves as one of the control measures to protect both the animals and the workers from unwanted microorganisms (Lipman & Leary, 2015:1547–1548).

The facilities are generally divided into two main areas, the dirty and clean areas, with a communal area, namely the cage wash area. This ensures that the dirty cages and their contents don't contaminate any areas that should remain clean. The physical layout of the facility is designed in such a manner that it follows a one-directional flow, from clean to dirty, to the wash area, and then enter the clean areas again (Figure 2.1). This is done to ensure that no contamination can occur from the dirty to the clean area (Lipman & Leary, 2015:1546).

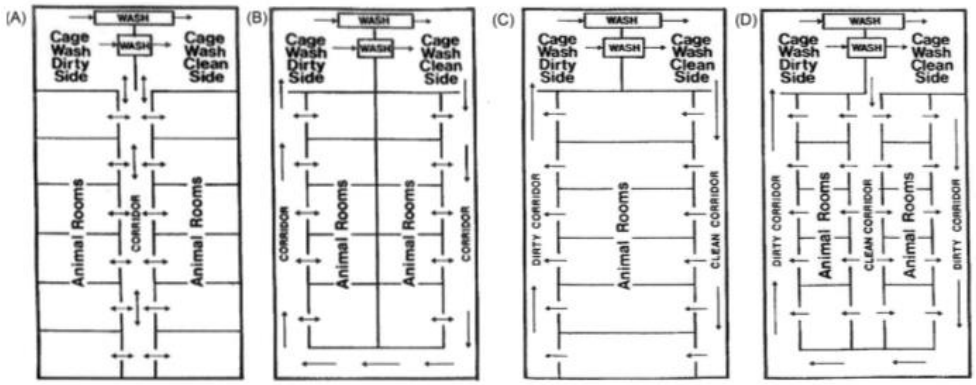


Figure 2.1: Four examples of different flow patterns that focus on the cage washing area. (A) Single-corridor bidirectional pattern; (B) single-corridor unidirectional pattern; (C) dual-corridor pattern with relatively large animal holding rooms; and (D) dual-corridor pattern with relatively small animal holding rooms. Source: Lipman and Leary, 2015:1546.

2.3.2 Animal bedding

Mayeux *et al.* (1995:2297) found that facilities in the United States of America that made use of bedding that was not heat-treated before use had fungal contamination that affected the test animals. This could lead to contamination of the workers as well because these spores can become airborne as soon as the bedding is used, and the workers can inhale those spores. They found mostly spores of *Penicillium ssp*., *Fusarium ssp*., *Cladosporium ssp*., *Rhodotorula ssp*., *Cryptococcus uniguttulatus* and *Aspergillus fumigatus*. This leads to the need for testing the bedding and feed before they can be used in the facility to ensure that no contaminated products enter the facility.

Kaliste *et al.* (2004:25) investigated the contamination of the animal bedding after it was used by animals, as well as the amount of bedding particulates that become airborne when being handled before and after use. They found that, depending on the type of bedding, the amount of bedding particles that became airborne decreased after it was used in the animal cages. The amount of airborne particulate concentration after the bedding was used ranged from < 1 to 8.6 mg/m^3, a major decrease from a concentration of < 1 to 25 mg/m^3, where the bedding was unused. They tested the aspen chip bedding commonly used by mice, rats and rabbits and found that the maximal

concentrations of bacteria, fungi and endotoxins were > 6 500 000 CFU/g, 212 000 CFU/g and 6 500 ng/g, respectively. This indicated that the used bedding is a large source of exposure to HBAs when not properly handled or disposed. The implications of improper disposal must be noted and the impact that can have on the environment and communities surrounding the facility. (Kaliste *et al.*, 2004:25). Kaliste *et al.* (2004:25) emphasised that the bedding material itself is a major source of airborne microbial exposure when workers handle, change, or dispose the bedding, and therefore handling procedures and appropriate disposal are critical to mitigate risk.

Due to lack of any more research on rodent bedding contamination, recent work outside rodent research has shown broad relevance of bedding materials as microbial reservoirs and dust sources. For example, a systematic review by Gomes *et al*. (2022:1) in poultry production under the One Health approach found that, of all environmental matrices assessed, the bedding material was the most contaminated and frequently harboured both bacterial and fungal species, with *Penicillium* spp. and *Aspergillus* spp. dominant among the fungal contaminants (Gomes *et al*., 2022:1). The review also noted that bedding materials contribute to airborne contamination in high-density animal housing and may serve as exposure sources for animals, workers and potentially the surrounding environment.

Additionally, Ray *et al*. (2022:1) examined unused and used bedding on commercial dairy farms and demonstrated that the microbial composition of bedding changes significantly after animal use. They found that bacterial counts, overall microbial diversity and the presence of environmental and zoonotic organisms increased substantially in used bedding. This indicates that bedding does not merely act as a passive substrate but develops into a biologically active material capable of harbouring and amplifying microbial communities, many of which can become airborne during handling. More recently, Rapp *et al*. (2023:1) found that, in dairy-calf housing systems, the storage and pre-use condition of bedding influence the bacterial community structure and the persistence of zoonotic bacteria such as *Escherichia coli* O157 and *Salmonella* spp. (Rapp *et al*., 2023:1). They suggested that bedding management before, during and after animal use is a significant factor in reducing zoonotic dissemination from bedding materials.

Collectively, these findings indicate that bedding in animal-housing environments is more than just a comfort or absorbent substrate: it is a biological reservoir that can generate airborne contaminants through particle agitation, microbial growth and spore release (Gomes *et al*., 2022:1; Kaliste *et al*., 2004:25). In the context of animal testing facility work, this means that inadequate pre-treatment, poor handling protocols, and inappropriate disposal of bedding materials may present significant occupational exposures to HBAs (Kaliste *et al*., 2004:25). Consequently, regular microbial testing of bedding and feed, stringent handling procedures, engineering controls during bedding change-out, and protocols for disposal are essential to protect both animals and workers (Gomes *et al*., 2022; Ray *et al*., 2022:1).

2.3.3 Personal protective equipment requirements

Personal protective equipment (PPE) should be provided by the animal testing facility to both the workers and visitors before they enter the facility. Guidelines developed specifically for animal research facilities similarly emphasise that PPE selection must consider the risks posed by biological, chemical and allergenic exposures (NIH, 2022:1–3). Examples of PPE include laboratory coats, sleeves, gloves, overalls, hair bonnets, shoe covers and a variety of face masks and respirators. This is done to protect the workers as well as the animals in the facility. It is, however, noted that the majority of the PPE used in these facilities is to protect the animals from possible outside contamination (Department of Employment and Labour, 2022:14–19; Lipman & Leary, 2015:1588–1589). An example of this includes the more common use of surgical masks instead of respirators. A surgical mask's main purpose is to stop droplets produced by the worker from either sneezing, coughing or talking from entering the surrounding environment. However, it does not protect the wearer from inhaling airborne pathogens that can be found in the surrounding area, as it does not form a tight seal around the nose and mouth area (Coffey *et al*., 2020). This limitation is consistently highlighted in institutional guidelines for animal research. The National Institutes of Health (NIH) notes that surgical or procedural masks "do not provide respiratory protection against airborne hazards" and should not be used where aerosolised biological material may be encountered (NIH, 2022:4). Similarly, the University of Michigan Environmental Health and Safety office states that surgical masks are *not* considered respirators under occupational health requirements and therefore do not offer meaningful protection against airborne contaminants (University of Michigan, 2025).

This distinction becomes especially important when workers handle animals, dirty cages or contaminated bedding, where airborne particulates, allergens and micro-organisms are routinely generated. According to Rengasamy *et al*. (2004:345), proper respiratory protection must be selected based on bioaerosol behaviour and exposure risk, as filtering efficiency varies greatly between surgical masks and certified respirators. Facilities such as the University of California, Irvine further specify that workers handling contaminated bedding or materials must receive appropriate respiratory protection, fit testing and training where airborne exposures may occur (University of California, 2024). If appropriate PPE is not used or safety measures are not followed, workers may be exposed to HBAs and could develop occupationally acquired infections (Davidson *et al*., 2020:493). Together, these findings demonstrate that PPE in animal testing facilities serves a dual purpose, and that worker protection must be considered with the same seriousness as the protection afforded to research animals.

2.4 Laboratory accidents

Ross and Harper (2023:1–2) highlighted the impact that biolaboratory accidents can have on the world. They stated that laboratory accidents pose serious and potentially catastrophic consequences, either by infections acquired due to occupational exposure or biocontainment breaches. Accidents leading to the spread of dangerous pathogens pose a threat to more than just the human population but to animals and plants as well, endangering economies, biodiversity, ecosystems, food security and the livelihood of communities (Dhawan *et al*., 2025:1). Despite measures being put into place to strengthen the controls when working with pathogens, regular and avoidable laboratory accidents still occur, while the number of laboratories and pathogen research projects keeps increasing. Ross and Harper (2023:3–4) investigated scientific literature, and official reports stretching over 21 years were reviewed. It was found that 94 incident reports were documented, which involved 309 individuals infected with lab-acquired infections and 51 pathogens of varying degrees of hazards. Some of these infections resulted in fatalities, and some spread to the community. It was also found that there were 16 incidents where biocontainment facilities had breaches. It was found that most of these incidents occurred in research or university laboratories. Even though reporting of occupational infections has decreased over time due to improved safety standards, increased awareness of biological hazards and improvements in technology, these findings were concluded to be an underestimation of the true value of incidents since many incidents go unreported (Wurtz *et al*., 2016:1247).

It has been found that the main causes of laboratory accidents are due to inadequate training, incorrect type or use of PPE or containment equipment, and the use of improper techniques or mishandling of specimens. Needle-stick injuries, contact with body fluids, animal bites and scratches, suspended aerosol inhalation, as well as engineering failures due to lack of maintenance or equipment malfunctioning, were also found to be significant causes of released pathogens that later resulted in infections. It was noted that nearly 70% of known cases were due to procedural errors that can easily be corrected, such as inadequate training, incorrect or inconsistent use of PPE, improper handling techniques, needle-stick injuries, and equipment misuse. The second highest at 9.1% was recorded as ‘unknown’, leading to a lack of understanding and failure to identify the exact causes of accidents (Ross & Harper, 2023:1–2).

It is important to look at case studies where accidental exposure to HBAs occurred. Specifically, to adapt risk assessments, PPE, training of workers, update relevant regulations and standards, as well as vaccination protocols to mitigate the risk of accidental exposures in the future (Rim & Lim, 2014:43)

Laboratory workers in Marburg and Frankfurt in Germany and Belgrade in Yugoslavia, now known as Serbia, mysteriously developed an epidemic of haemorrhagic fever in 1967, leading to a total of 32 patients that included laboratory workers, medical personnel and family members, where seven of

those cases turned out to be fatal. The source of this mysterious infection was traced back to kidney cells that were extracted from African green monkeys from Uganda, which were acquired by all three locations for poliomyelitis research. The Marburg virus, named after the city with the most cases, has since been involved in several laboratory accidents and subsequent outbreaks with a death rate of up to 90% (Brauburger *et al*., 2012:1878–1879).

An unusual epidemic of anthrax occurred in April and May of 1979 in Sverdlovsk, Russia. The Soviet officials claimed it was from consumption of contaminated meat. However, United States agencies linked it to an accidental release of spores from a military facility in the city. The leak of the spores were linked to procedural and engineering failures and led to 64 deaths and several animal infections, of which the number is unknown (Meselson *et al*., 1994:1202).

After the global pandemic of SARS-CoV-2, Demaneuf (2020:1–25) investigated severe acute respiratory syndrome (SARS) reappearances after the end of the 2003 epidemic. It was found that three cases were connected to laboratory accidents in Singapore, Taiwan and China. The Singapore case originated from a university laboratory and was caused by improper experimental procedures. A 27-year-old university student who had no prior experience with BSL-3 laboratories was given access to one after undergoing training on BSL-3 procedures for approximately 20 minutes. The student, however, did not follow proper protocol and was not supervised when working with the SARS samples. The student tested positive a few days later and made a full recovery with no secondary infections occurring. The Taiwan incident occurred at a military medical research laboratory where exposure was traced to the improper handling of a leaking bag containing virus samples in a negative-pressure transport cabinet. The researcher was on his way to a conference in Singapore when he noticed the spill. Due to him being in a rush, he could not find the correct PPE or have time to clean the spill properly. He could not adequately reach the spill and decided to insert his head into the cabinet to reach the spill and clean it with 70% ethanol, which is improper protocol. He still decided to travel internationally and came into contact with a total of 100 people, where 95 could be contacted and instructed to self-isolate for 21 days. Due to his travelling while in the non-contagious phase, no secondary infections were recorded. The Chinese incidents consisted of two separate incidents. The first was of a student who followed an unsuitable protocol to deactivate the virus, ignorant of the fact that the protocol was unsuccessful; the student studied the virus and got infected, as well as one other person working in the same area. Both recovered, and no secondary infections were recorded. Another student from the university, working in another part of the facility that had no contact with the SARS laboratory, contracted the virus from an unknown source. Secondary infections occurred while the student was treated medically and a total of nine cases were recorded with one resulting in death (Demaneuf, 2020:1–25).

The United Kingdom had an outbreak of bovine foot and mouth disease in 2007. It was determined that the most likely cause of the outbreak was from the government's Institute for Animal Health in Pirbright, Surrey, which leaked contaminated wastewater. The contamination was caused by an improper procedure to render the materials inactive that were stored in a bulk holding tank. The effluent was then discarded through drainage pipes that were damaged and old, leaking and contaminating the surrounding soil. The contaminated soil was then aerosolised and spread to nearby farms when vehicles drove over it, acting as transportation. The disease spread to nearby farms, leading to a national livestock movement and trade ban and the culling of 2 000 animals (Anderson, 2008:8–13).

A laboratory in China was the source of a large-scale outbreak of brucellosis, a zoonotic disease, in 2019. It was found that the facility used expired disinfectants that led to the release of viable aerosolised pathogens into the air. The pathogens were carried by the wind to nearby communal settlements and academic institutes. More than 10 000 people tested positive for the bacteria (Pappas, 2022:1845).

2.5 HBA sampling methodologies

There is a lack of standardised procedures for the sampling of hazardous biological agents in animal testing facilities, particularly for bioaerosols, despite the variety of sampling methods available. This gap arises because sampling in these environments requires consideration of facility-specific factors, such as animal species and stocking density, patterns of animal handling, ventilation design, cage-changing and cleaning activities, and differences in biological waste management. It must also account for the general variables of sampling frequency, duration, location, sampler type and downstream analytical techniques (Whitby *et al.,* 2022:109). Thus, it is important to consider the advantages and disadvantages of each method and what type of information is being requested. Whitby *et al.* (2022:146) investigated all available bioaerosol sampling techniques and noted all the advantages and disadvantages of these methods.

Active bioaerosol sampling refers to techniques that actively draw a known volume of air through or onto a collection medium using a powered sampling device. These methods are designed to provide a more representative assessment of airborne microbial exposure, particularly with respect to inhalation, as they are not reliant on gravitational settling of particles. Active sampling enables the quantification of microorganisms per unit volume of air and is therefore commonly used in occupational and indoor air quality investigations where airborne exposure is of concern (Whitby *et al.*, 2022:109–118).

Different active sampling technologies vary in their collection principles, particle-size efficiency, suitability for culture-dependent or culture-independent analysis, and their ability to capture viable microorganisms. As a result, the choice of active sampler should be guided by the study objectives, expected bioaerosol concentrations, and the type of downstream analysis required (Whitby *et al*., 2022:109–123). Standards and guidance documents also emphasise selecting and verifying samplers based on purpose, considering physical/biological collection efficiency and exposure conditions, when designing microbial air monitoring in controlled environments (ISO, 2003).

2.5.1 Microbial air sampler

The MAS-100 NT®, classified as an International Organization for Standardization (ISO) 14698 portable microbial air sampler (MAS), is an impactor that deposits the sampled bioaerosols directly onto the agar plate using forced air movement (Millipore, 2020:1). This instrument is convenient and easy to use. It allows for direct collection that leads to reduced post-sampling efforts and errors. It also allows for the total sample to be examined without any need for extraction or sub-sampling. However, due to the high flow, the particles may not adhere to the surface of the agar plate, known as particle bounce, and smaller particles with low inertia will be carried by the streamline and not be captured on the plate. There is also a possibility of microbial overload on the plate, resulting in inaccurate enumeration and/or difficulty in obtaining isolates (Whitby *et al.,* 2022:118). A study was conducted in Denmark at two different biowaste pretreatment plants using various biological sampling techniques, including Gesamtstaubprobenahme (GSP) filter samplers for environmental and personal sampling, one- and six-stage Anderson Cascade Impactors as well as the MAS100 instrument as short-term statutory sampling methods (Rasmussen *et al.*, 2021:273). Sampling times for the MAS100 ranged from 0.5 to 3 minutes to minimise the risk of overloading the plates due to the high microbial load in the biowaste plants. Rasmussen *et al.* (2021:237) reported that the impaction methods during the shortest sampling period (30 seconds) taken during unloading of waste (by waste collection workers) were in the order of 10^3 CFU/m^3. They also reported that the personal sampling methods (GSP) that sampled for times ranging between 60 minutes and 429 minutes, exposure to bacteria and fungi averaged in the order of 104 CFU/m^3, which provided workable results. The results from the impaction methods (MAS and Anderson) were, however, excluded from the study due to unreliable results and were only provided in a separate supplementary information document. Another study was done at nine salmon processing plants in Norway to assess airborne bacterial and fungal species (Madsen *et al.*, 2024:1–2). The MAS100 instrument was used with agar plates specific to the growth of fungi and various types of bacteria, and the sampling duration ranged from 1 to 2.5 minutes per plate. The studies showed that the overall bacteria and fungi concentrations were low, but had a high bacterial species richness, where human and fish pathogens and food spoilage bacteria were found. Concluding from both these studies that the MAS100/MAS-100 NT® is a good instrument to quantify

bioaerosol presence in places where not high concentrations are expected to minimise the risk of overloading the plate.

2.5.2 Bioaerosol cyclone

The TE-BC251 bioaerosol cyclone is a sampler that can either be used for personal or area sampling and is not dependent on a culture medium. The sampler includes two empty disposable microcentrifuge tubes (1.5 and 15 ml), as well as a 0.8 µm pore size mixed cellulose ester (MCE) membrane filter. The sampler makes use of centrifugal force to capture particles of specific sizes in each stage of the sampler. The first larger tube captures particles of 4 µm and larger, the second smaller tube captures particles from 1 to 4 µm, and the filter captures particles smaller than 1 µm (NIOSH, 2020:1). It is a gentle and low-stress sampling method, and a film of liquid can be injected near the inlet of the sampler to increase the efficiency by having a liquid medium that can be analysed. The sampler is, however, dependent on a calibrated air flow for accurate collection efficiency. It is also more likely to capture larger particles than smaller particles, leading to under-sampling of smaller bioaerosols (Whitby *et al.,* 2022:120). There is a lack of literature specifically regarding the use of this instrument, however, there are studies where other devices in this line was modified to act the same as this specific sampler. Lee and Liao (2013:725) conducted a study where a two-stage bioaerosol cyclone was modified to act as a three-stage bioaerosol cyclone by adding an external filter cassette to the sampling train. The cyclone was used to assess size-selective fungi and fungal fragments of farmers working on corn, poultry, mushrooms and swine farms. The study found that most of the fungi particles sampled were larger than 1.8 µm and only a few particles were smaller than 1 µm. It was speculated that most of the smaller particles were fragments released when the larger particles were airborne. This causes concern due to certain dangerous fungi that are known as large particles that can fragment into smaller particles that will then be able to penetrate deeper into the lungs when inhaled.

2.5.3 Settle plates

Settle plates are a passive sampling method that makes use of gravity to capture settling particles directly on the agar plate. It is an easy sampling method to use. It can provide the total number of collected bioaerosols and can be used to identify the bioaerosols in the building or room by using specified agar plates. It is, however, non-quantitative and settling dust particulates can contain non-aerosolised sources and could lead to difficulty in interpreting the data (Whitby *et al*., 2022:122–123). A clinical ward experienced an outbreak of Group A *Streptococcus* (GAS) among their patients with no obvious site of infection and it was hypothesised that the spread may be due to an infected health care worker (Mahinda *et al*., 2018:209). Thirty blood agar settle plates were deployed within various areas of the clinic and the health care workers' common areas. Sampling was performed over seven

days, and the plates were exposed to the air for a total of four hours each day. The plates were incubated at 37°C for 24 hours whereafter colonial morphology confirmed the presence of GAS. The study highlighted five out of the thirty areas that had positive results for two days or more, with the staff kitchen being one of them. Additional medical surveillance sampling of the health care workers identified one nurse who tested positive for GAS during throat and perineal swabbing. Additional safety and preventative measures could be implemented using the information gathered (Mahinda *et al*., 2018:209). Another study was conducted at a dry-cured meat facility that compared the SAS−super−180 air sampler and settle plates assessment of fungal particles within the air of the facility (Asefa *et al.,* 2008:997). The settle plates were used applying the 1/1/1 scheme, where the plates are placed for one hour at a height of one metre off the ground and one metre away from any walls or obstacles. The plates were placed in duplicate within the rooms. All the sampled plates were incubated for seven days in the dark at 25 ± 1°C and identified using macro and microscopic morphological characteristics. The results showed that the air sampler yielded a higher number of species and mean CFU/plate when compared to the settle plates. However, the two methods showed similar dominating fungal genera and species that are associated with the dry-cured meat process and products. This study highlighted the fact that settle plates could provide qualitative information, specifically in this study, for food processing plants where airborne fungi that are associated with food products, are the targets.

2.5.4 Surface swabbing

Surface swabbing is a technique widely used to collect any microbial particulates that can be found on a flat even surface area, this includes airborne particles that have settled on the surface (Lindsley *et al.,* 2017:25). Viegas *et al*. (2023:4–9) conducted a study in grocery shops in Portugal and Spain using both surface swabbing and electrostatic dust cloths (EDCs). EDCs are cloths that are electrostatically charged and designed to pick up dust without causing it to resuspend in the air (National Allergy, 2025). The results obtained in Portugal showed that the surface swabs had the highest average of bacterial contamination, as well as the highest CFU of Gram-negative bacteria when compared to that of EDCs. In Spain, the EDC yielded the highest median value; however, what was interesting was that the total median number of bacterial count was lower than that of Portugal in EDC samples. The surface swabs in Portugal also showed the highest fungal count compared to that of EDCs. It was also observed that the surface swabs had a higher abundance of *Aspergillus* species than that of the EDCs. Their study indicates that surface swabbing provides the best results for surface sampling compared to EDCs.

2.5.5 Surface contact sampling

Contact plates are agar plates poured in a specific way to form a convex shape over the rim of the plate so that when the agar is pressed against an uneven surface, it can pick up any microbes directly onto the agar (Lindsley *et al.*, 2017:27). Claro *et al*. (2015:1000–1002) conducted a pilot study comparing the use of contact plates to that of Petrifilms by sampling contaminated hospital surfaces. Petrifilms are a food-safe alternative surface sampling method that removes the need to make agar plates (Selectech, 2025). Claro *et al*. (2015:1000–1002) sampled surfaces such as the toilet door handles, bedside lockers, tray tables, and call buttons in the morning and the evening. As predicted, the toilet door handle was, on average the most contaminated surface and surprisingly, the Petrifilms detected the highest colony forming unit (CFU) concentration from all surfaces compared to that of the contact plate samples. They did, however, state that more research was needed to make a definitive conclusion regarding the use of standards and methods when it comes to the specific sampling method of uneven surfaces.

2.5.6 Environmental factors that may influence the presence of hazardous biological agents

Environmental conditions such as temperature, carbon dioxide (CO_2) concentration and air flow should also be recorded as well as the occupancy and activity in the rooms, as these factors could influence the microbial presence in the room or areas. Kalyoncu (2010:553–557) conducted a study assessing the relationship between environmental factors and fungal allergens in a city in Turkey. The study was conducted over two years. Kalyoncu (2010:556) found that there is a correlation between the presence of airborne fungal allergens and meteorological factors such as temperature ($r = 0.6$) and relative humidity (RH) ($r = 0.7$). It was found that the colony count and total fungal genera were the lowest in the months with the lowest and highest temperatures; the lowest temperatures ranged between -5.8°C and -24.8°C and the highest temperatures ranged between 9.6°C and 40.4°C. The optimal temperature was found to be in the month where the temperature ranged between 9.5°C and 32.4°C, where the colony count and the total fungal genera were almost double of that during the lowest and highest temperatures. However, with regard to RH, the study showed that as the RH increased, so did the colony count and the total fungal genera, with the highest readings at the RH of 97.7% to 80% and the lowest readings at the RH of 23% to 24%. The study further showed that the effects of wind speed did not influence the colony count or total genera that stayed within the same ranges, with the lowest wind speeds at 10.8 m/s and the highest at 25 m/s. It was concluded from this study that the optimal temperature for fungal growth is between 20°C and 24°C, and optimal RH is > 65%.

These observations are supported by several additional studies. Sabariego *et al*. (2000:1) found that airborne fungal spores in Spain showed clear seasonal peaks linked to high temperatures and

increased RH, demonstrating strong meteorological influence on fungal levels. Indoor studies also confirm these trends. Li and Kendrick (1995:190) demonstrated that indoor fungal levels increased with elevated RH, inadequate ventilation and increased human activity, highlighting that occupancy and movement can influence airborne fungal concentrations. Grinn-Gofroń and Bosiacka (2015:63) also showed that mean air temperature and RH were the most important meteorological variables explaining variation in airborne fungal spore composition, further supporting the strong influence of environmental conditions on fungal presence in the air.

These results were further confirmed by De Ligne *et al*. (2019:1), who assessed fungal growth of *Coniophora puteana* and *Rhizoctonia solani* with 16 combinations of temperatures (15, 20, 25 and 30°C) and RH (65, 70, 75 and 80% RH). They found that the optimal environmental conditions for *R. solani* growth were at 20°C, 75% and 25°C, 80%; and 20°C, 75% for *C. puteana.* These results prove that environmental conditions have a direct influence on the total airborne fungal allergens.

2.6 Oxford nanopore sequencing

Deoxyribonucleic acid (DNA) sequencing is a laboratory technique used to determine the sequence of nucleotides/bases (adenine (A), cytosine (C), guanine (G) and thymine (T)) in a DNA molecule. The biological information that cells use to develop and operate is encoded in the sequence of the DNA bases, and DNA sequencing is done to understand the function of genes and other parts of the genome (Adams, 2025). Sequencing is also used to identify species and provide information on the abundance and richness of microorganisms (EPI2ME, 2020; ONT, 2025b). In microbiome research, two complementary strategies are commonly applied: marker-gene amplicon sequencing (for example, 16S ribosomal ribonucleic acid (rRNA) for Bacteria/Archaea and Internal Transcribed Spacer (ITS) for Fungi) and shotgun metagenomics, which sequences the total DNA in a sample (Jovel *et al.*, 2016:1–3; Usyk *et al.*, 2023:1–2). Amplicon surveys are widely used for cost-efficient assessments of community structure and alpha/beta-diversity, whereas shotgun metagenomics provides broader genomic context and direct access to functional genes (Jovel *et al.*, 2016:1; Usyk *et al.*, 2023:1). Comparative evaluations across cohorts show that amplicon and shotgun outputs often agree at the genus level, while shotgun sequencing can improve recovery of low-abundance taxa and enables functional inference. Consequently, the approach selection should align with aims, biomass and matrix constraints (Usyk *et al.*, 2023:2–5; de Vries *et al.*, 2023:1–2; Liao *et al.*, 2023:1–2).

Each strategy has well-characterised methodological considerations. Amplicon data are sensitive to primer choice, PCR conditions and the analysis pipeline; reproducible, provenance-tracking workflows such as QIIME 2 combined with denoising (for example, DADA2) help reduce error and enhance transparency (Bolyen *et al.*, 2019:852–856; Callahan *et al.*, 2016:581–582). Shotgun metagenomics typically requires deeper sequencing and greater computational resources; widely

used tools include Kraken 2 for fast k-mer classification with Bracken for abundance re-estimation, and Minimap2 for long-read alignment where applicable (Wood *et al.*, 2019:1; Li, 2018:3094). These choices influence taxonomic assignment and abundance estimates and should be selected in conjunction with sampling design and the intended ecological or exposure-assessment questions; harmonisation across strategies is feasible, especially at genus level, when validated workflows and consistent metadata practices are used (Usyk *et al.*, 2023:4–6). Within host-associated biomes, community approaches have clarified when amplicon versus shotgun is most informative. A methodological review using clinical gut datasets illustrated that amplicon surveys remain efficient for diversity and compositional trends, whereas shotgun data enable direct interrogation of metabolic pathways and strain-level features; critically, both strategies can be harmonised for meta-analysis when consistent processing and metadata standards are applied (Jovel *et al.*, 2016:1; Usyk *et al.*, 2023:4–6).

In engineered and built environments, community-level sequencing has been used to track system performance and potential exposure points. For instance, parallel 16S rRNA amplicon and shotgun metagenomics in household on-site wastewater treatment systems showed that both approaches produced comparable overall profiles at moderate abundance but that shotgun sequencing identified additional low-abundance taxa and provided functional insights relevant to process optimisation and risk appraisal (de Vries *et al.*, 2023:1–3). Similarly, comparative work on wildlife-associated biomes found that amplicon and shotgun outputs aligned at genus level, while shotgun offered finer taxonomic discrimination of putative pathogens of interest for environmental health surveillance (Liao *et al.*, 2023).

In occupational and environmental settings, particularly low-biomass air or surface samples where host DNA and background contaminants are common, combining culture-dependent approaches with culture-independent, sequence-based community profiling yields a more complete picture of exposure (de Vries *et al.*, 2023:2–3). Within this framework, long-read sequencing adds value by spanning full-length marker genes (for example, 16S rRNA), improving species-level resolution, and enabling portable, real-time metagenomic characterisation in field and low-biomass contexts (Szoboszlay *et al.*, 2023:1; Veselovsky *et al.*, 2025:1). Nanopore-based approaches have already been applied to air-microbiome investigations, demonstrating feasibility for species-resolved aerosol profiling, while constant improvements in chemistry and basecalling continue to increase performance, which should nevertheless be matched to application-specific accuracy requirements (Reska *et al.*, 2024:1). Air microbiome studies have begun to exploit nanopore-enabled metagenomics to generate species-resolved community profiles directly from bioaerosols. In a recent application spanning greenhouse, outdoor and urban sites, nanopore-based metagenomics, coupled with tailored computational workflows, resolved core airborne taxa and site-specific signatures,

demonstrating feasibility for near-real-time community characterisation in low-biomass matrices that are typical in occupational settings (Reska *et al.*, 2024). Such air-biome characterisation complements culture-based monitoring by revealing the broader community context in which HBAs occur.

Oxford Nanopore Technologies (2025b) developed a unique method that enables direct, real-time analysis of short to ultra-long fragments of DNA without the need for polymerase chain reaction (PCR) amplification, which can help to provide access to time critical information. The Oxford Nanopore sequencing devices, such as the MinION, make use of flow cells which contain an electro-resistant membrane embedded with an array of nanopores (Figure 2.2).

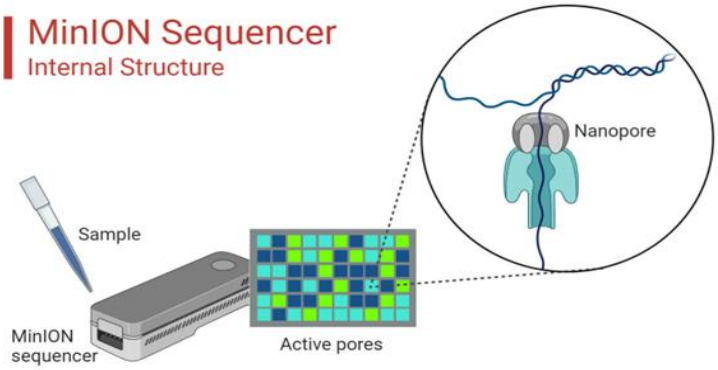


Figure 2.2: Visual illustration of the MinION device nanopores (Tamang, 2024).

The nanopores correspond to their own electrodes connected to channels and sensor chips that measure the electrical current that flows through the nanopore. A ‘squiggle’ is produced when the current is disrupted by the molecule passing through the nanopore. The basecalling workflow is then used to decode the squiggle and provides the DNA sequence for further processing depending on the researchers' need (ONT, 2025a) (Figure 2.3).

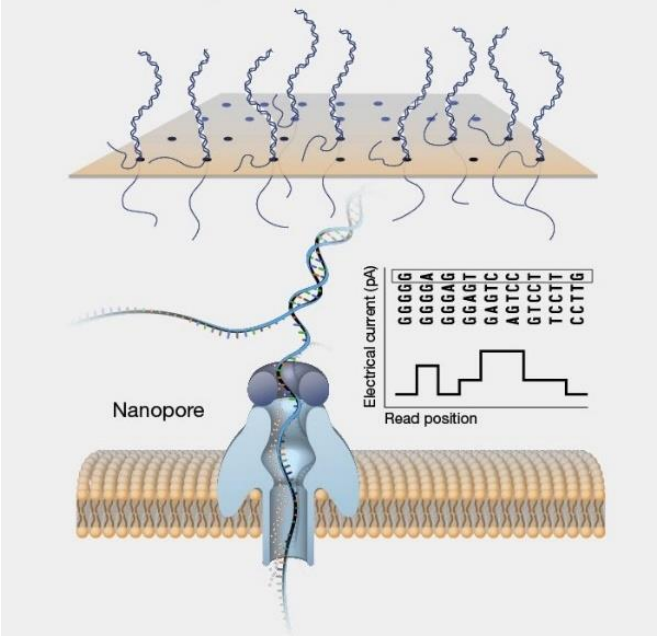


Figure 2.3: Visual illustration of nanopore sequencing and basecalling (Adams, 2025).

Marshall *et al.* (2024:754) compared the MinION nanopore sequencing method to the more conventional method, the sequencing of a PCR-amplified target gene, known as the Sanger method. The Sanger method is usually the default method due to the affordable and accurate results that it offers; it is, however, limited to single species and cannot identify multiple species microbial colonies. The study made use of seven distinct and randomly selected bacterial colonies obtained from bioaerosol samples that were cultured on tryptic soy agar (TSA). It was found that both techniques indicated the presence of the same bacterial genus in five out of the seven samples. For the two remaining samples, the first colony provided a noisy Sanger electropherogram that failed to generate any meaningful results, where the nanopore sequencer identified a mix of two bacterial genera, *Alkalihalobacillus* and *Kocuria*. For the second colony, the Sanger method yielded a clean electropherogram that suggested the genus *Micrococcus*, and the nanopore sequencing method suggested the genus *Paraburkholderia* as an additional presence in the colony. The findings of these tests were corroborated by the use of mock multispecies colonies from pure bacterial DNA samples. The results led to the conclusion that there are distinct differences between the nanopore sequencing and Sanger method (only five out of seven samples obtained similar results). This is expected, as Sanger sequencing produces accurate results but is limited to sequencing a single amplicon and therefore cannot resolve multiple species when they occur in the same colony. In contrast, nanopore sequencing generates long reads and detects a wider range of DNA present in mixed samples, allowing it to identify individual bacterial components within multispecies colonies. This characteristic could be advantageous for colony identification of culture-based environmental samples, such as

bioaerosols. Szoboszlay *et al*. (2023:1) reported that, within the context of their simulation work conducted on gut microbiota from human faecal samples, nanopore sequencing provided broader species-level resolution than Illumina sequencing for 16S rRNA gene amplicons, particularly when aiming to detect rare taxa or obtain a more accurate richness estimate. However, the study also recommends using Illumina 16S sequencing for microbial communities with many unidentified species or when the goal is to resolve amplicon sequence variants, as the higher per-base accuracy and lower error rate of Illumina platforms enable more reliable discrimination of closely related sequences (Szoboszlay *et al*., 2023:1).

2.7 Concluding remarks

Hazardous biological agents remain a significant occupational health concern, particularly in work environments where contact with animals, organic material and biological waste is unavoidable. The literature demonstrates that animal testing facilities represent a unique exposure scenario, as workers may be exposed to HBAs through multiple routes, including inhalation of bioaerosols, contact with contaminated surfaces and materials such as animal bedding, and direct interaction with animals. Although these facilities are designed to protect research integrity and animal health, the protection of workers against biological hazards may not always receive the same attention.

Further examination of literature highlights that there is no single standardised method for assessing exposure to HBAs in animal testing facilities. Culture-dependent sampling methods remain widely used due to their simplicity and cost-effectiveness, yet they are limited by their inability to detect non-culturable microorganisms. Passive sampling methods, such as settle plates and surface sampling, provide valuable information on surface contamination and microbial fallout but cannot quantify airborne exposure. Active air sampling, while more representative of inhalation exposure, may still underestimate microbial diversity when used in isolation. These limitations emphasise the need for a combined sampling approach when assessing occupational exposure to HBAs.

Advances in genomic analysis, particularly Oxford nanopore sequencing, have introduced the opportunity to overcome several of the shortcomings associated with traditional culture-based methods. Nanopore sequencing allows for the identification of a broader range of microorganisms, including non-culturable species, and enables taxonomic classification at genus and species levels. When applied alongside conventional microbiological methods, this approach provides a more comprehensive understanding of the microbial environment and potential occupational risks.

In summary, the literature indicates a clear gap in published data regarding the presence and identification of hazardous biological agents in animal testing facilities, particularly within the context

of occupational exposure assessment. The absence of exposure limits for HBAs further complicates risk evaluation, placing greater importance on the identification of agents and their classification into risk groups. This study addresses these gaps by combining passive and active bioaerosol sampling with genomic analysis to characterise HBAs within an animal testing facility and to evaluate the potential occupational exposure risks faced by workers.

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Proverbs 3: 5 – 6

Trust in the Lord with all your heart, and do not lean on your own understanding.

In all your ways acknowledge Him, and he will make straight your path.

CHAPTER 3 ARTICLE

The article is written in accordance with the guidelines of the NWU Harvard Style and follows the United Kingdom English spelling with exceptions made for the names and references used.

General principles: The referencing list must start on a new page. All sources must be sorted into alphabetical order according to the author. “Bullets” or numbering must not be used, and the line spacing must be 1.5 and the spacing must be aligned and not justified. Published sources must be italicised and all entries must have a full stop at the end except when a DOI, permanent internet link or hyperlinked URL is used.

Examples:

Jakobson, R. 1959. On linguistic aspects of translation. In: Venuti, L., ed. 2004. *The translation studies reader.* 2nd ed. New York, NY: Routledge. Pp. 138-143

Schreurs, B., De Cuyper, N., Van Emmerik, I.J.H., Notelaers, G. & De Witte, H. 2011. Job demand and resources and their association with early retirement intentions through recovery need and work enjoyment. *South African Journal of Industrial Psychology*, 37(2), art. #859. doi:10.4102/sajip.v37i2.859

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Hazardous biological agents and the occupational exposure risks thereof in an animal testing facility

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3.1 Abstract

Animal testing facilities play a vital role in the world of biomedical research. However, the presence of hazardous biological agents (HBAs) introduces potential health risks for personnel involved. This risk arises from possible exposure to HBAs through inhalation of urinary proteins and faecal matter from the animal's bedding, animal dander or accidental animal bites and scratches. Exposure to HBAs could lead to a variety of negative health effects that include inflammatory responses, respiratory diseases, digestive tract infections and general discomfort. This study aimed to determine the presence and identify potential HBAs in an animal testing facility. Active and passive biological sampling were performed using both culture-dependent methods, such as active air sampling on agar plates, settle plates, surface swabs, and contact plates and culture-independent methods, including the bioaerosol cyclone, a size-selective sampler. Metabarcoding was carried out using nanopore sequencing to identify potential HBAs present in the facility at both the genus and species levels. Furthermore, physical parameters were measured to assess their influence on the abundance of microbial growth, while chemical parameters (dust particulate matter) were measured to evaluate their role as carriers and the potential risk of HBA inhalation. A total of 62 HBAs were identified in the facility, five of which are toxin-producing, and 53 classified as bacterial risk group 2 agents according to the Regulations for Hazardous Biological Agents, 2022 (South Africa). Six HBAs were identified as fungal risk group 2 agents, and three risk group 3 agents. The highest average concentration of microbial growth was in the cloak room collected by use of the microbial air sampler. The high microbial concentrations in the cloak room indicate that the area, which is supposed to be clean when donning and doffing personal protective equipment (PPE), is actually a hazard area and a source of contamination for the workers in this area and the rest of the facility. There were no statistically significant correlations between the environmental parameters and the microbial growth quantified within the facility. The combination of low air velocity and a high concentration of small dust particles (< 1 µm) suggests that the smallest particles (potentially containing HBAs) remain suspended in the air for extended periods, increasing the risk of inhalation and deep lung penetration. The variety of risk group 2 and 3 HBAs present in the facility and inadequacies of the control measures, such as the PPE and poor ventilation systems, lead to the conclusion that the HBAs found in the animal testing facility pose a potential moderate risk to its workers' health.

Key words: Metabarcoding, health risk assessment, microbial contamination, occupational exposure risk

3.2 Introduction

A hazardous biological agent (HBA) is defined as an “agent which may cause infection, allergy or toxicity or otherwise create a risk to human health” (Department of Employment and Labour, 2022:4). HBAs are some of the most challenging hazards to assess in the field of occupational hygiene due to the limited knowledge on monitoring and risk to health estimation (Davidson *et al.,* 2020:488). HBAs are also not that well-known or defined compared to the physical and chemical agents within the field of occupational hygiene; this could be due to the broad range of HBAs with their various and complex effects on human health (Van den Heever, 2014:199–200). HBAs encompass a diverse range of agents, such as potentially infectious viruses, bacteria, fungi, parasites, archaea and protozoa. They can also consist of products of plants, insects, animals, including cell-wall chemicals, prions, fungal spores, toxins and fragments, dust mites, animal and insect dander, plant fibres, grain, pollen and organic dust (Davidson *et al.,* 2020:488). Therefore, it can be assumed that HBAs can be found everywhere in our everyday life in one way or another.

Due to the large variety of HBAs, many can be grouped using a common similarity as a denominator. Examples of these types of HBAs are coliforms and fastidious bacteria. Fastidious bacteria are notoriously difficult to grow, as they need perfect conditions and nutrition to stay alive. Blood agar is commonly used as a growing agent due to its added nutrition and haemolytic properties, which can in turn help identify the pathogenicity of the bacteria. The bacteria secrete cytolytic toxins, leading to haemolysis (the destruction of the red blood cells in the agar) creating a colour change around the bacterial colonies, which in turn indicates its potential pathogenicity. Examples of these types of bacteria include *Legionella pneumophila*, *Bordetella pertussis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* (Aryal, 2022). Coliforms are bacteria that can ferment lactose with the production of acid and gas at temperatures between 35°C and 37°C and are most commonly found in the aquatic environment. Coliforms themselves may not necessarily cause serious illness, but their presence is used to indicate that other pathogenic organisms of faecal origin may be present. Such pathogens include disease-causing bacteria, viruses, or protozoa and many multicellular parasites (Li & Liu, 2019:303–305). One of the most well-known coliforms is *Escherichia coli* (*E. coli*). These two categories of bacteria are important to note as they were specifically tested for in this study using selective agar.

Exposure to HBAs follows three main routes, namely, inhalation of aerosolised particles, ingestion (vehicle transmission) and dermal contact, including needle stick injuries, cut accidents and animal and insect bites (vector transmission) (Figure 3.1). It is essential to keep this fact in mind when dealing with HBAs in the work environment, as the route of exposure could help to determine control measures to mitigate the risk of exposure (Davidson *et al.,* 2020:492).

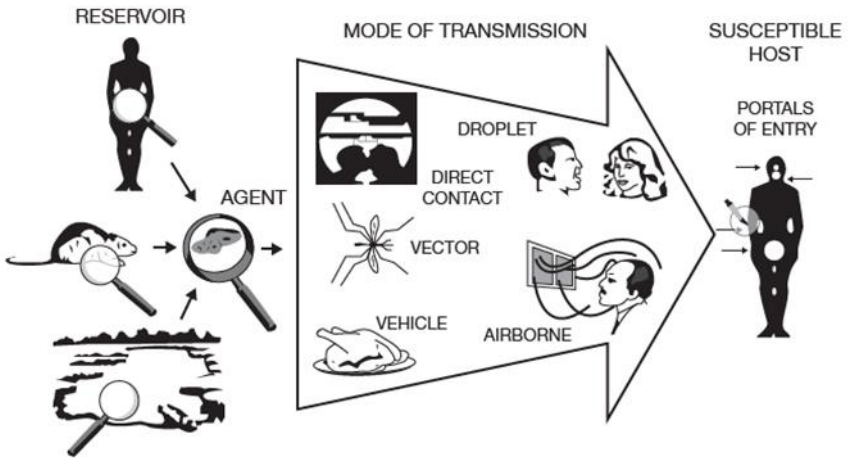


Figure 3.1: Exposure routes for hazardous biological agents (Davidson *et al.,* 2020:492).

Exposure to HBAs could lead to a variety of occupational diseases such as infectious or respiratory disorders, skin conditions and even cancer. For example, Hepatitis B and some types of Human Papillomavirus (HPV) can increase the risk of specific cancers (Stanley, 2017:1). A large range of HBAs can also cause allergic reactions such as allergic alveolitis, contact dermatitis and occupational asthma (Davidson *et al.,* 2020:490–491). Infectious diseases, also known as communicable diseases, originate either from humans, animals or contaminated food or water. Zoonoses are diseases that are specifically from the transmission of pathogens from animals to humans and include all infectious biological agents. Respiratory diseases can be divided into allergic and non-allergic conditions, where an allergic response is the activation of the adaptive immune system by a foreign substance and a non-allergic disease is caused by irritation, innate immune-system response or mechanical blockage (Davidson *et al.,* 2020:488–505). With regard to cancer-causing HBAs, the International Agency for Research on Cancer (IARC) has a list of agents and a classification of carcinogenicity, and this list includes certain HBAs. These types of agents include aflatoxins that are caused by *Aspergillus ssp*., a Group 1 carcinogen, and the malaria parasite, a Group 2A carcinogen (IARC, 2024). These occupational illnesses are significant when evaluating HBAs in the workplace.

Animal testing facilities are laboratories where research is conducted using animals. These types of facilities include universities and government-funded research facilities with the research topics ranging from pharmaceutical development to biomedical research (Leszczynski, 2018). The exposure potential to HBAs in these facilities is unique compared to other workplaces where animals are worked with, due to the possible exposure to known pathogenic organisms. The guidelines for these laboratories are known as biosafety level (BSL), and there are four different levels (Princeton University, 2025). The specific facility in this study is a BSL-2 facility, meaning that basic personal protective equipment (PPE), such as lab coats and surgical gloves, is worn, and additional PPE, such

as hair bonnets, shoe covers, and surgical masks, is included to protect the animals and the research. The entire facility is connected to a mechanical ventilation system, which is thoroughly controlled, not making use of any natural ventilation. Animal testing facilities do, however, follow strict rules regarding safety, specifically against the pathogens that are being experimented on and researched in the facility. Laboratory accidents are also more common than they should be because they could have catastrophic consequences to the workers and the surrounding environment (Ross & Harper, 2023:1–2). While safety measures are implemented, such as personal protective equipment in certain facilities, they tend to prioritise the protection of the research over the safety of the workers leading to a unique exposure scenario where all routes of exposure are of concern (Lipman & Leary, 2015:1588–1589).

The unique environment presented in the animal testing facilities provides multiple potential exposure pathways to HBAs for workers. There is a risk that the worker may inhale urinary proteins and faecal matter from the bedding, or aerosolised animal food and water particulates, experience bites and scratches from the animals, as well as exposure to the environmental conditions within the building (Van den Heever, 2014:199–200). There are many possible areas where accidental cross-contamination can occur from the animals to the workers. This means that the HBAs found in an animal testing facility can be harmful to human health if exposure is not correctly controlled or monitored (Davidson *et al.*, 2020:490–491). This study aimed to determine the presence and identify hazardous biological agents within an animal testing facility.

3.3 Materials and methods

3.3.1 Facility description

This study was approved by the North-West University's Health Research Ethics Committee (HREC) (NWU-00097-24-A1). A walk-through survey was conducted to familiarise the researchers with the facility layout, control measures and potential exposure sources. The facility operates on a clean-dirty separation system, with movement restricted to prevent re-entry into clean zones after entering dirty areas. Ventilation is provided exclusively by a centralised heating, ventilation, and air conditioning (HVAC) system that regulates temperature, relative humidity and maintains positive pressure. Basic PPE, including laboratory coats and disposable gloves, is required, with additional PPE used where necessary to protect research integrity.

Based on the survey, the following areas were selected for sampling (Figure 3.2). The cloak room functions as the transition point between clean and dirty areas and houses the required PPE. The experimental room serves as the primary research space; however, only an inactive experimental room was accessible for sampling due to restrictions on entering active study areas. The breeding

room contains approximately 280 animal cages and serves as the main housing and breeding area for animals used in experiments. The facility hallways connect clean and dirty areas, with movement rules in place to minimise contamination between areas. The washing area, located in the basement, contains cage-washing equipment, an autoclave and laundry facilities for laboratory coats. This area receives dirty cages and bottles from the upper floors for cleaning and sanitisation. During the survey, dirty bedding was observed in a biohazardous waste bin in the washing area, indicating a potential source of HBA exposure. Please see supplementary material for a more in depth discussion of each room sampled in the facility.

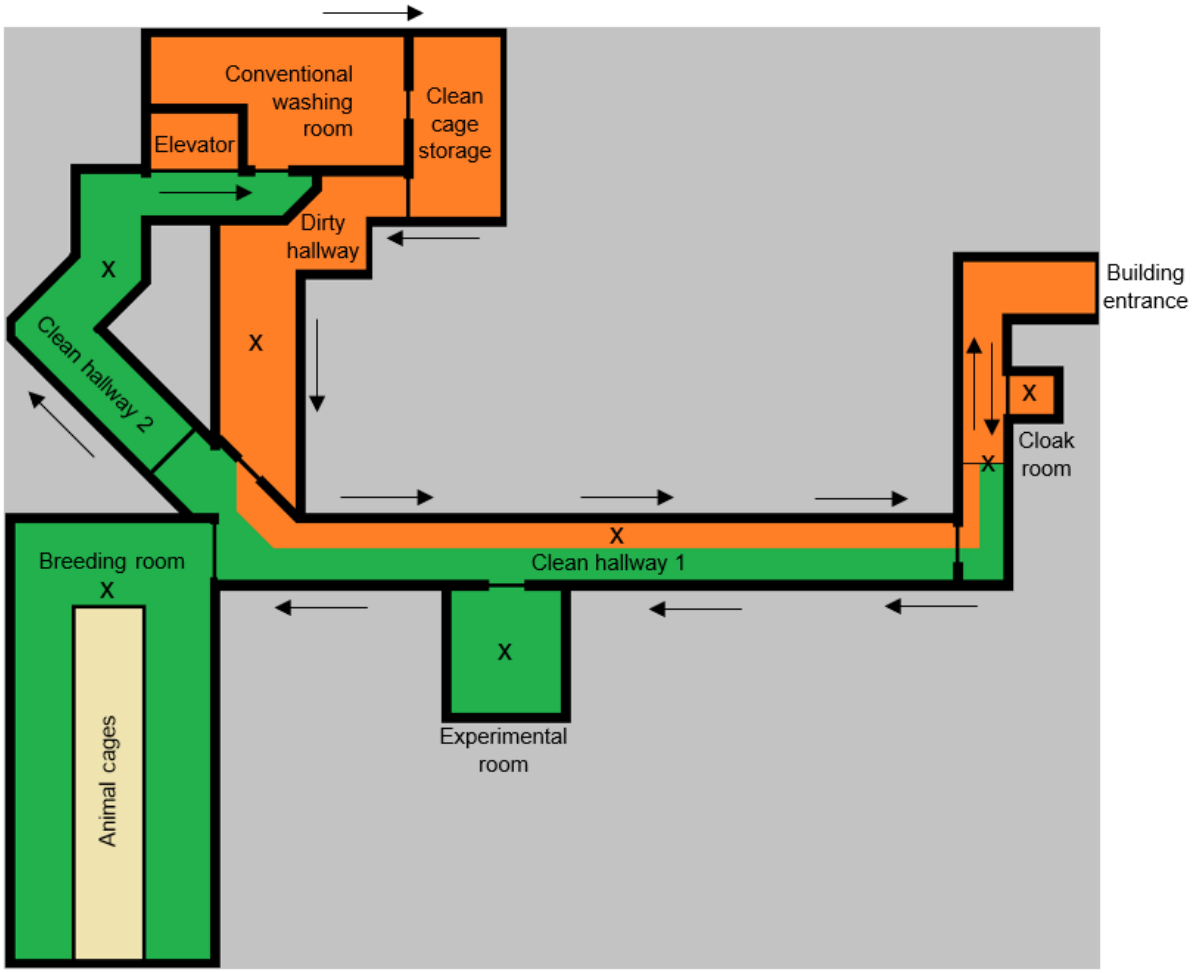


Figure 3.2: The floor plan of the animal testing facility. Green indicates the clean areas, and orange indicates the dirty areas. Sample collections are indicated with an 'x' and the arrows show the flow when moving through the facility. Grey indicates the rest of the facility's experimental rooms, offices, storage rooms and maintenance rooms, which were not included in the sampling.

3.3.2 Sampling

Sampling of the facility was conducted over three days: Monday, Wednesday and Friday within one week. During sampling, three different types of samples were collected, namely chemical, biological and physical (Figure 3.3). All detailed protocols and extended methodological information are available in the supplementary material (Chapter 5).

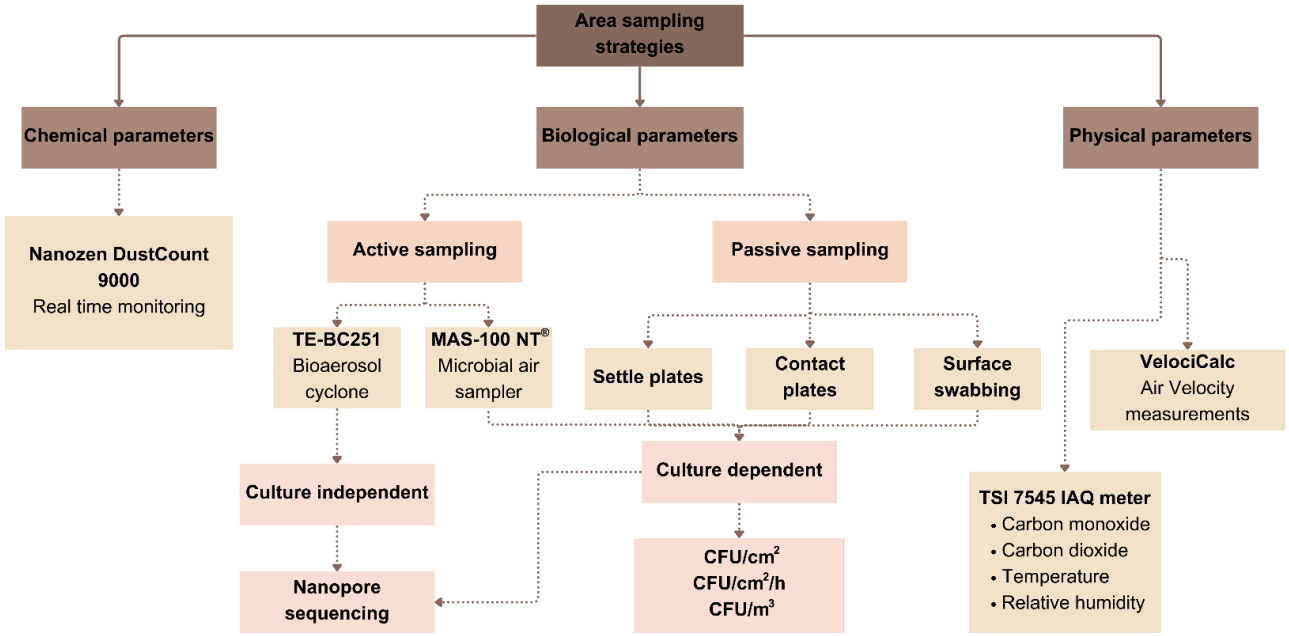


Figure 3.3: Workflow of sample collection methods followed in this study.

3.3.2.1 Passive sampling

Passive environmental sampling for HBAs included surface swabs, contact plates, and settle plates. Surface swabbing was conducted on workbenches, PPE cupboards, floors, washbasin areas, and air supply ventilation openings using sterile 10 x 10 cm templates where possible, with swabs applied in a systematic pattern to maximise coverage (Figure S.1). Contact plates were used on irregular surfaces such as washbasins, extraction vents, animal cages, door handles, and lab coats, while settle plates were positioned in hallways to capture airborne microorganisms for 1–1.5 hours. All samples were labelled, transported to the laboratory, and incubated at 37°C for 48 hours following standard protocols (Hanlon & Hodges, 2013:18; Lindsley *et al*., 2017:23–24).

3.3.2.2 Active sampling

Airborne microbial sampling was performed using a MAS-100 NT® microbial air sampler and a TE-BC251 bioaerosol cyclone. The MAS-100 NT® was pre-calibrated to operate at 100 L/min for 10

minutes and was positioned on workbenches in various rooms to capture inhalable bioaerosols directly onto agar plates (Millipore, 2020). The TE-BC251 cyclone collected size-specific particulates (>4 µm, 1–4 µm, and <1 µm) using a three-stage system and was mounted on a tripod approximately 1 m off the ground, operating for 45 minutes per location (NIOSH, 2020:1–2). All equipment underwent rigorous sterilisation, including ethanol treatment and autoclaving where applicable, and sampling accessories were assembled under aseptic conditions. After collection, agar plates were incubated at 37°C for 48 hours, while cyclone filters and tubes were stored frozen for subsequent sequencing (Hanlon & Hodges, 2013:18).

3.3.2.3 Environmental parameters

Environmental parameters were assessed using three instruments: the Nanozen DustCount 9000 for real-time dust particulate matter concentration and size distribution (Nanozen, 2019:12–15), the TSI IAQ-Calc™ 7545 for carbon monoxide (CO) concentrations, carbon dioxide (CO_2) concentrations, temperature, and relative humidity (TSI, 2024:6–16), and the TSI Airflow™ TA465-P for air velocity (TSI, 2022:3–7). The DustCount operated at 1 L/min for approximately 45 minutes per area, while IAQ and airflow measurements were taken in all locations, with averages calculated from three to four readings per site. All instruments were cleaned and calibrated according to manufacturer protocols prior to use. Data were recorded on field sheets and downloaded for analysis.

3.3.3 Plate counting and analysis

The agar plates were removed from the incubator after the 48−hour incubation period and stored in a fridge overnight to cease growth. The plates were then removed, and the visible colonies were counted by the researcher to obtain the total colony forming units (CFUs). This was done by using a black marker to mark each colony on the bottom of the plate while counting, ensuring that no colony was missed or accidentally counted twice (Schulze *et al.*, 2024). The blood agar haemolysis was classified as either alpha (α), beta (β), or gamma (γ) growth. Alpha haemolysis shows a partial breakdown of the red blood cells, resulting in a greenish discolouration around bacterial colonies. Beta haemolysis causes total lysis of red blood cells, leaving a clear zone surrounding the colony. Gamma haemolysis indicates growth with no haemolysis, meaning the agar remains unchanged and opaque (Burton & Lalande, 2021).

3.3.4 Metabarcoding

Deoxyribonucleic acid (DNA) was extracted from selected agar plate growth and cyclone sampler filters and tubes using the NucleoSpin® Soil kit (Macherey-Nagel, 2024) following manufacturer instructions. Samples were selected based on growth abundance and morphology, grouped by sampling type, room, and day, and processed in a laminar flow cabinet. DNA quality and concentration

were assessed using a NanoDrop UV-Vis Spectrophotometer (Thermo Fisher Scientific, 2023:25–27), and extracts were stored at −20°C until sequencing could be conducted. Nanopore sequencing was performed using the Rapid Sequencing DNA V14 Barcoding Kit (SQK-RBK114-24) and MinION MK1B device (ONT, 2025). Barcoded samples were pooled and prepared into a sequencing library before loading onto a primed FLO-MIN114 flow cell. Sequencing was conducted using MinKNOW software (version 6.2.14).

3.3.5 Data analysis

3.3.5.1 Microbial growth calculations

The CFU counts were imported into Microsoft Excel spreadsheets (version 2504), where the CFU concentrations were calculated using the formulas listed in Table 3.1.

3.3.5.2 Nanopore sequencing data analysis

The sequencing procedure produces raw data signals when the electrical current is disrupted as the molecule passes through the nanopore. The raw signal output data was provided in the form of POD5 files. These files were run through the basecalling workflow, which decodes the raw signals and provides the sequence of bases within the strands of the DNA. Dorado is the default basecaller integrated within the MinKNOW application. This data is then stored in FASTQ files (ONT, 2025). The FASTQ files are run through the metagenomic workflow, using the EPI2ME application (version 5.2.3). The metagenomic workflow uses Kraken2 and Minimap2 software to facilitate the taxonomic classification of sequence reads from metagenome samples. Both Standard-8 and ncbi_16s_18s_28s_ITS databases were used to run the metagenomic workflow. A final report was provided with information pertaining to the taxonomy, abundance and alpha diversity indices (EPI2ME, 2020).

The observed index measures the number of different species detected in a sample. A higher value means more species were present (Gotelli & Colwell, 2001:379). The Shannon diversity index measures the diversity of species by combining richness and evenness of a sample; the higher the value, the higher the diversity and a value that equals zero indicates only one species in the sample (Kers & Saccenti, 2022:3–4). The Simpson's index measures how evenly species are distributed in a sample. It considers both the number of species and how dominant each one is. Lower values indicate higher diversity, while higher values show that only a few species dominate (Magurran, 2021:1175).

The differences in microbial community composition between samples were calculated using the Bray–Curtis dissimilarity metric, and these differences were visualized using Principal Coordinates Analysis (PCoA). PCoA based on Bray–Curtis dissimilarity was used to compare microbial community

composition between samples. In a PCoA plot, points that cluster closely represent communities that are more similar, while points that are further apart indicate greater dissimilarity. The axes (PCoA1 and PCoA2) show the main dimensions of variation, with their position reflecting relative similarity rather than abundance (Ramette, 2007:148).

Table 3.1: Formulas used to calculate the total colony forming units (CFU) per surface area for each of the sampling methods.

Sample collection method	Calculations	Reference
Surface swabbing (surface area of the template)	$\frac{\text{CFU}}{\text{Surface area sampled}} = \text{CFU/cm}^2$ $\frac{\text{CFU}}{100\ \text{cm}^2} = \text{CFU/cm}^2$	(ISO, 2018)
Contact plates (surface area of the agar on the contact plate)	$\frac{\text{CFU}}{\text{Surface area sampled}} = \text{CFU/cm}^2$ $\frac{\text{CFU}}{23.75\ \text{cm}^2} = \text{CFU/cm}^2$	(ISO, 2018)
Settle plates (surface area of the agar in the plate)	$\frac{\text{CFU}}{\text{Surface area sampled}} \div \text{h} = \text{CFU/cm}^2\text{/h}$ $\frac{\text{CFU}}{63.62\ \text{cm}^2} \div \text{h} = \text{CFU/cm}^2\text{/h}$	(Viani *et al.,* 2020)
Microbial air sampler*	$\frac{\text{CFU}}{\text{Total volume of air sampled}} = \text{CFU/m}^3$ $\frac{\text{CFU}}{1\ \text{m}^3} = \text{CFU/m}^3$	(Sivasankar, 2016)

* A Feller formula-based, MAS-100 positive hole conversion table, supplied by the manufacturer was used to acquire the CFU values for the microbial air sampler (Millipore, 2020). The MAS ran for 10 minutes at a flow rate of 100 L/min for each sample. This resulted in 1000 L of air being drawn through the sampler, which is equal to one cubic metre of air sampled.

3.3.5.3 Statistical analysis

The biological, physical and chemical parameter data were imported into Microsoft Excel spreadsheets to calculate the means and standard deviations. The means and standard deviation values were then used to calculate the z-scores, determining any outliers in the data that may influence the interpretation of the results. Z-scores above 3 and below -3 were considered outliers (Chikodili *et al.*, 2021:265).

Statistical analysis was done to investigate a possible correlation between physical and chemical environmental factors, and the microbial growth in the animal testing facility. All physical parameters

were correlated with all sampling methods in all areas of the facility, and the dust particulate matter was correlated with the MAS samples of each area, as both methods are active air sampling methods.

The statistical analyses were performed using GraphPad Prism (version 10.5.0 (774)). A Shapiro-Wilk test was done to assess the distribution of the data (normal or non-normal) (Malato, 2023). Data sets identified as non-normally distributed were log-transformed and re-tested using the Shapiro-Wilk test. Some data sets were still identified as non-normal leading to the analysis of the data using a non-parametric test, specifically by calculating a Spearman rank correlation coefficient. The test provides the correlation coefficient (r) and the corresponding p-value. The correlation coefficients were interpreted as: r = 0–0.19 as very weak, r = 0.2–0.39 as weak, r = 0.4–0.59 as moderate, r = 0.6–0.79 as strong and r = 0.8–1 as very strong. All the p-values were considered statistically significant where $p \leq 0.05$ (BMJ, 2020). Statistical comparisons between the different sampled areas within each room were also conducted. Due to the non-parametric nature of the data, a Kruskal-Wallis test was performed to evaluate whether there were significant differences in microbial concentrations across various sampled areas. Where the Kruskal-Wallis test yielded statistically significant results ($p < 0.05$), Dunn's multiple comparisons post hoc test was used to identify specific pairwise differences between the groups (McDonald, 2014:158–164; Ruxton & Beauchamp, 2008:690).

3.4 Results

3.4.1 Microbial growth throughout the facility

The following section reports on the concentrations of microbial growth collected using different sampling methods within the different areas of the facility over three days. The results presented in the figures are grouped according to the sampling media used and the mean of the results over three sampling days was calculated. Non-specific bacteria were grown on nutrient agar (NA), yeast and moulds on potato dextrose agar (PDA), coliforms on Endo agar and lastly, the fastidious bacteria were grown on blood agar.

3.4.1.1 Surface swabbing

3.4.1.1.1 Cloakroom

Surface swabs were taken throughout the facility to detect contamination on regular surfaces. Within the cloak room, the specific sampled items included the shelves of the cupboards housing clean PPE as well as the clean and dirty sides of the floor when entering and exiting the facility (Figure 3.4). A For non-specific bacterial growth, the mean ranks indicated that the clean floor exhibited the highest concentrations (mean rank = 6.6), while the PPE cupboard showed the lowest levels (mean rank = 2.0); however, a Kruskal-Wallis test with Dunn's multiple comparisons confirmed that these differences were not statistically significant ($p > 0.1$) (Figure 3.4 a). An outlier was observed in the clean floor non-specific bacterial data on the second day with a concentration of 13.69 CFU/cm² (z-score = 5.90) (individual values are not shown). For yeast and mould growth, mean ranks suggested higher concentrations on the dirty floor relative to the other sampled areas, and this was supported by the statistical analysis, which showed a significant difference between the dirty floor and PPE cupboard samples ($p = 0.02$), while no significant differences were observed between the PPE cupboard and clean floor ($p = 0.8$) or between the clean and dirty floors ($p = 0.3$) (Figure 3.4 b). For coliform bacteria and fastidious bacteria, the descriptive mean ranks showed minor variation across the sampled areas, but the Kruskal-Wallis tests demonstrated no statistically significant differences between any of the locations (Figure 3.4 c; $p > 0.9$) (Figure 3.4 d; $p > 0.5$). The fastidious bacteria plates yielded growth with beta haemolysis on the dirty floor the first day and PPE cupboard and clean floor on the third day (results not shown).

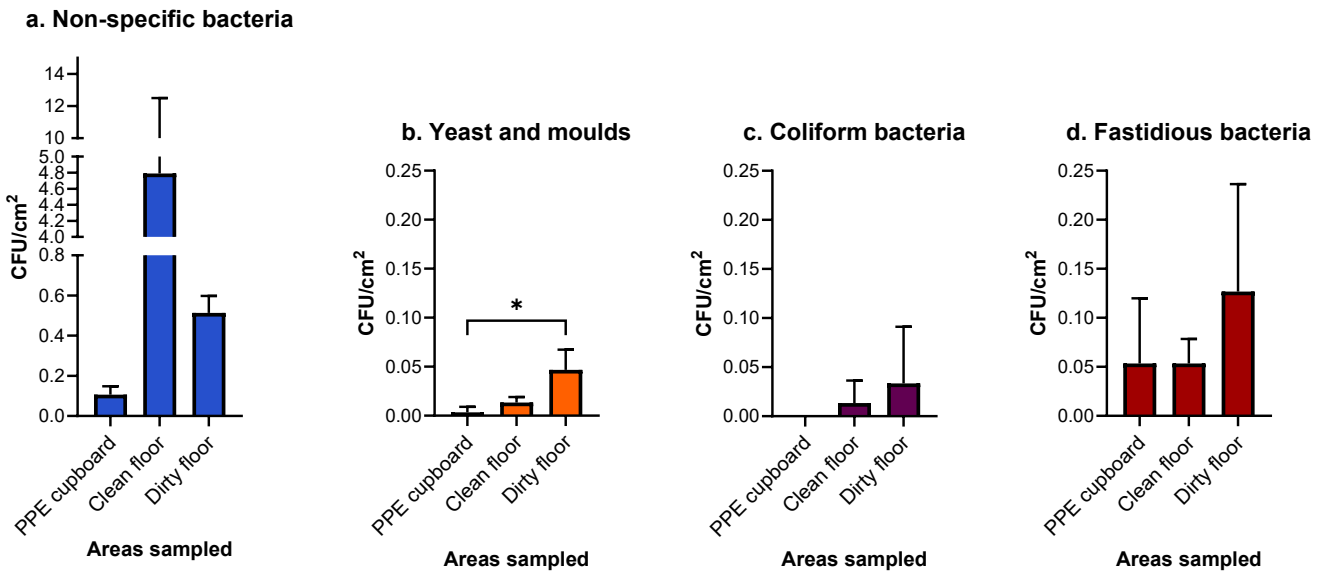


Figure 3.4: Mean CFU/cm^2 of surface swabbing in the cloak room, on four different growth media, over three days. (a) Non-specific bacteria; (b) yeast and moulds; (c) coliform bacteria, and (d) fastidious bacteria. Bar graph indicates the mean, and error bars indicate the standard deviation. The horizontal line with an asterisk (b) denotes a statistically significant difference between the PPE cupboard and dirty floor (p = 0.02).

3.4.1.1.2 Experimental room and hallways

Almost no growth was observed in the experimental room and hallways. The air supply ventilation in the experimental room and hallways was swabbed. Swabs for fastidious bacteria were taken on the first day, and due to no microbial growth, samples were not collected on days two and three. The only growth that occurred in the experimental room was on the second day with 0.02 CFU/cm^2 yeast and mould growth (results not shown). Results from all three hallways yielded no growth except for the third day, with 0.01 CFU/cm^2 and 0.31 CFU/cm^2 of non-specific bacterial growth in clean hallway 1 and clean hallway 2, respectively (results not shown).

3.4.1.1.3 Breeding room

The sampled surfaces in the breeding room included the air supply ventilation, the inside of a washbasin that had visible mineral salt buildup from tap water and a trolley that was occasionally used as a workstation when it wasn't used for transporting cages. The trolley was the only area chosen to be swabbed for fastidious bacteria (blood agar) due to the possible cross-contamination from the dirty bedding in the cages that were placed on the trolley. The air supply ventilation had no growth over the three days (Figure 3.5 a, b and c). Blood agar was specifically used to sample for pathogens,

which is why it was only applied to the trolley. Since this was the only area sampled with blood agar, no comparative statistics were calculated due to the lack of comparable areas. As a result, the graph (Figure 3.5 d) displays the concentrations for each day individually rather than summary statistics. The highest concentration of growth for the trolley was fastidious bacteria on the second day, with a concentration of 0.18 CFU/cm^2 (Figure 3.5 d) and the rest of the growth had concentrations < 0.1 CFU/cm^2. The fastidious bacteria plates yielded overall growth with alpha haemolysis on the first day as well as beta haemolysis on the second day. An outlier was noted with a concentration of 12.09 CFU/cm^2 non-specific bacterial growth on the inside of the washbasin, on the third day (z-score = 5.33) (individual results not shown). For all three of the growth media, non-specific bacteria, yeast and mould, and coliform bacteria, the mean ranks showed that the inside of the washbasin had the highest concentrations of microbial growth, followed by the trolley. However, despite these apparent differences, the Kruskal–Wallis test with Dunn's multiple comparisons demonstrated that none of the pairwise comparisons were statistically significant (all p > 0.2296) (Figure 3.5 a, b and c).

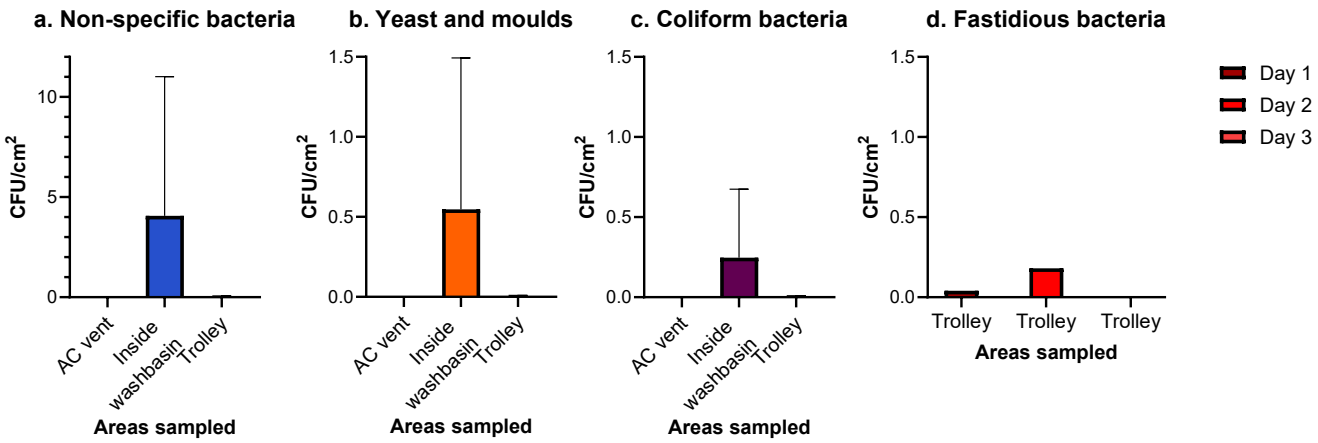


Figure 3.5: Mean CFU/cm^2 of fastidious bacteria surface swabbing in the breeding room, on four different growth media, over three days. (a) Non-specific bacteria; (b) yeast and moulds; (c) coliform bacteria, and (d) fastidious bacteria. Bar graphs indicate the mean, and error bars indicate the standard deviation.

Swabs from the clean bedding produced no growth over all four media (non-specific bacteria, yeast and mould, coliform- and fastidious bacteria) over all three days. Swabs from the dirty bedding had growth over the entire plate on all four media over all three days (results not shown). The fastidious bacteria plates yielded growth over the majority of the plate with alpha haemolysis over all three days (images of this growth can be found under supplementary material (Figure S4–5)).

3.4.1.1.4 Washing area

The surfaces sampled in the washing area consisted of an air supply ventilation, a stainless-steel bench used as a workstation for miscellaneous work near the entrance of the area, as well as a water change table which was primarily used to clean and refill the animals' water bottles, but also sometimes used as an extra workstation. The workstation was the only area chosen to be swabbed for fastidious bacteria (blood agar) due to it being the only workable surface and its proximity to the door, which could provide a source of HBAs from outside and other rooms. Also, a biohazardous waste disposal bin which contains laboratory paper towels and disposable gloves was swabbed, and dirty bedding was observed on certain days. Blood agar was specifically used to sample for pathogens, which is why it was only applied to the workstation. Since this was the only area sampled with blood agar, no comparative statistics were calculated due to the lack of comparable areas. As a result, the graph (Figure 3.6 d) displays the concentrations for each day individually rather than summary statistics. The fastidious bacteria plates yielded growth with alpha haemolysis on the first day (0.16 CFU/cm^2) as well as alpha and beta haemolysis on the third day (0.19 CFU/cm^2) (Figure 3.6 d). For non-specific bacterial growth, the mean ranks indicated higher concentration at the workstation compared to the water change table and air supply ventilation. The Kruskal-Wallis test with Dunn's multiple comparisons confirmed a statistically significant difference between the workstation and the air supply ventilation ($p = 0.03$), indicating greater microbial growth at the workstation (Figure 3.6 a). No significant differences were found between the workstation and the water change table ($p = 0.5$) or between the water change table and the air supply ventilation ($p = 0.7$). An outlier was observed in the water change table yeast and mould data on the third day, with a concentration of 2.27 CFU/cm^2 (z-score = 4.74) (individual results not shown). For yeast and mould growth and for coliform bacteria, the mean ranks showed minor variation between sampled areas, but the Kruskal–Wallis analyses indicated no statistically significant differences for either microbial group (all $p > 0.2$) (Figure 3.6 b and c).

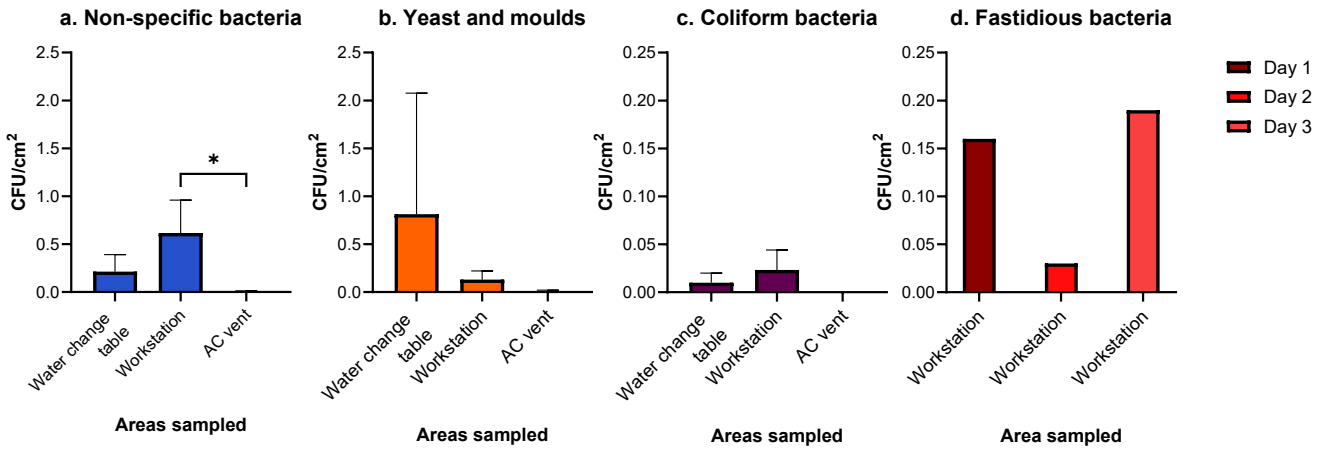


Figure 3.6: Mean CFU/cm^2 of surface swabbing in the washing area, on four different growth media over three days. (a) Non-specific bacteria; (b) yeast and moulds; (c) coliform bacteria, and (d) total fastidious bacteria. AC vent - air supply ventilation. Bar graph indicates the mean, and error bars indicate the standard deviation. The horizontal line with an asterisk (a) denotes a statistically significant difference between the workstation and AC vent (p = 0.03).

3.4.1.2 Contact plates

3.4.1.2.1 Cloak room

Contact plates were collected throughout the facility to detect contamination on irregular surfaces. The sampled surfaces in the cloak room included the clean lab coats in a storage bin, the outside of a cardboard box containing unused disposable surgical gloves and the door handle leading into the clean area of the facility (Figure 3.7 a, b and c). For non-specific bacteria and for yeast and mould, the mean ranks indicated that lab coats had the highest concentrations of microbial growth (mean rank = 7.3 and 6.3, respectively), followed by the glove box (mean rank = 4.6 and 5.8 respectively), with the door handle showing the lowest levels (mean rank = 3.0 and 2.8 respectively). However, the Kruskal-Wallis test with Dunn's multiple comparisons showed that these differences were not statistically significant (all p > 0.14). An outlier was noted of 2.19 CFU/cm^2 non-specific bacteria growth on the glove box, on the third day (z-score = 3.12) (individual results not shown). For coliform bacteria, the descriptive mean ranks similarly suggested higher growth on the lab coats (mean rank = 5.6), followed by the door handle (mean rank = 5.333) and the glove box (mean rank = 4.0), but these differences were also not statistically significant (p > 0.9).

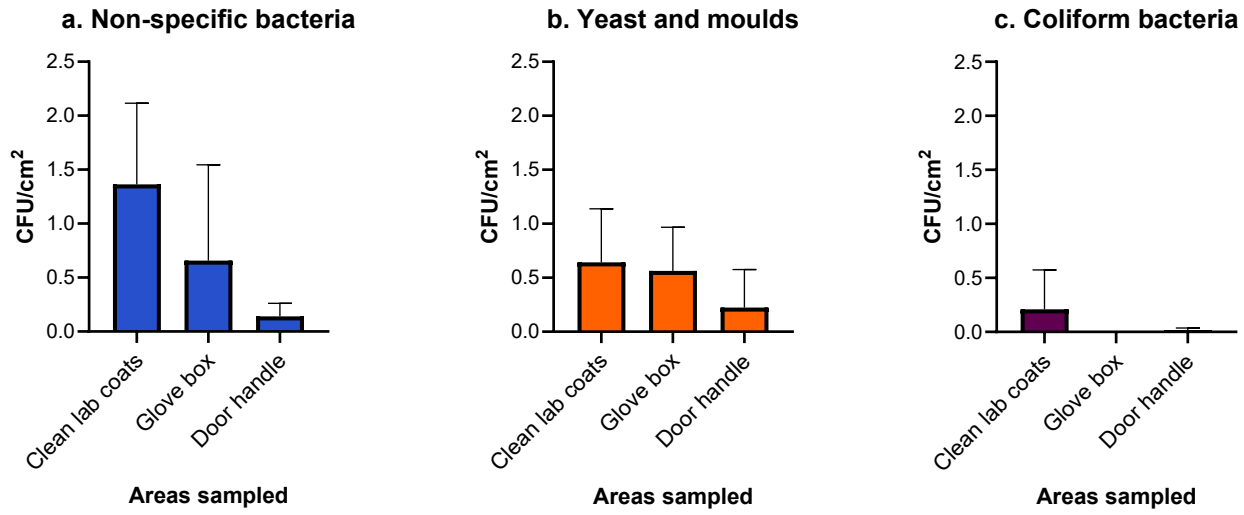


Figure 3.7: Mean CFU/cm^2 of contact plates in the cloak room, on three different growth media over three days. (a) Non-specific bacteria, (b) yeast and moulds, and (c) coliform bacteria. Bar graph indicates the mean, and error bars indicate the standard deviation.

3.4.1.2.2 Experimental room

The only sampled area using the contact plates in the experimental room was the extraction ventilation grate due to visible dust and dander buildup on the grate and the lack of activity in the room. As a result, the graphs (Figure 3.8 a and b) display the concentrations for each day individually rather than summary statistics. This data is displayed according to the concentration of growth per day due to only one sampled area. The highest concentration of growth was from non-specific bacteria growth on the second day with a concentration of 21.18 CFU/cm^2 (Figure 3.8 a), and the second highest was yeast and mould growth on the first day with a concentration of 19.24 CFU/cm^2 (Figure 3.8 b). There was no growth of coliform bacteria observed in this area over the three days. Day three had the highest concentration of growth from the non-specific bacteria (Figure 3.8 a). No outliers were noted in this area.

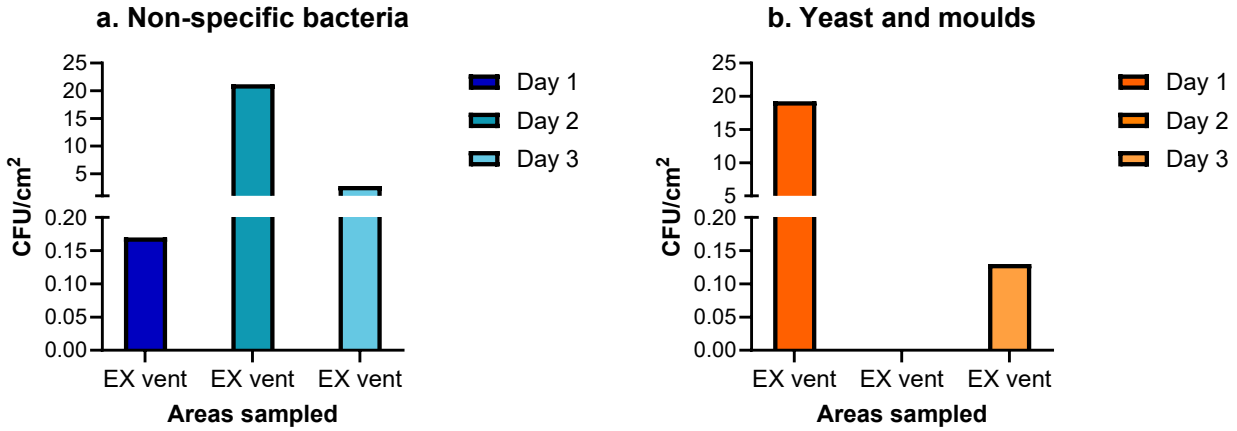


Figure 3.8: Total CFU/cm^2 of contact plates in the experimental room, on three different growth media, over three days. (a) Non-specific bacteria, and (b) yeast and moulds. EX vent - extraction ventilation grate.

3.4.1.2.3 Breeding room

The sampled surfaces within the breeding room included the irregular surfaces inside the washbasin, specifically around the drain area, the irregular curvatures of the trolley that was occasionally used as a workstation, as well as the outside surfaces of the occupied animal cages, which were regularly touched and handled (Figure 3.9 a, b and c). The trolley was only sampled from the second day due to an assumption of high growth and possible presence of pathogens due to work with animal bedding. An outlier was noted of 22.95 CFU/cm^2 coliform bacteria growth on the inside of the washbasin, on the third day (z-score = 3.11) (individual results not shown). For non-specific bacteria, yeast and mould, and coliform bacteria, the mean ranks showed that the washbasin had the highest concentrations of microbial growth (mean rank = 6.3, 6.3 and 7.0, respectively), followed by the trolley (mean rank = 3.5, 5.5 and 4.0, respectively), and then the animal cages (mean rank = 3.3, 2.0 and 2.3, respectively). However, the Kruskal-Wallis test with Dunn's multiple comparisons indicated that these differences were not statistically significant (all $p > 0.07$).

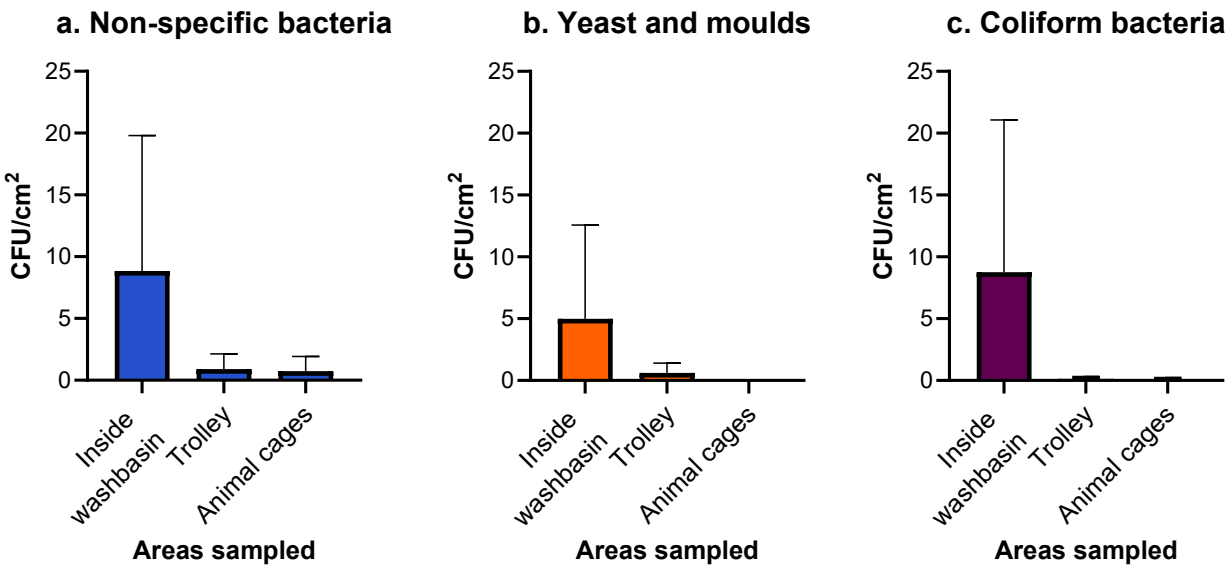


Figure 3.9: Mean CFU/cm^2 of contact plates in the breeding room, on three different growth media, over three days. (a) Non-specific bacteria, (b) yeast and moulds, and (c) coliform bacteria. Bar graph indicates the mean, and error bars indicate the standard deviation.

3.4.1.2.4 Washing area

The sampled surfaces in the washing area included the inside of a washbasin. The edge of the same washbasin was also sampled due to visible runoff from the cage cleaner that was observed. The other surfaces sampled were clean animal cages set aside to be stored in the clean cage storage in the main part of the facility. An outlier was noted of 42.57 CFU/cm^2 yeast and mould growth on the washbasin edge, on the third day (z-score = 4.87) (individual results not shown). For non-specific bacteria, yeast and mould, and coliform bacteria, the mean ranks indicated that the washbasin bowl had the highest concentrations of microbial growth (mean rank = 7.0 for all three), followed by the washbasin edge (mean rank = 5.3, 4.6 and 5.0, respectively), with the animal cages showing the lowest levels (mean rank = 2.6, 3.3 and 3.0, respectively) (Figure 3.10 a, b and c). However, the Kruskal-Wallis test with Dunn's multiple comparisons showed that none of these differences were statistically significant (all $p > 0.14$).

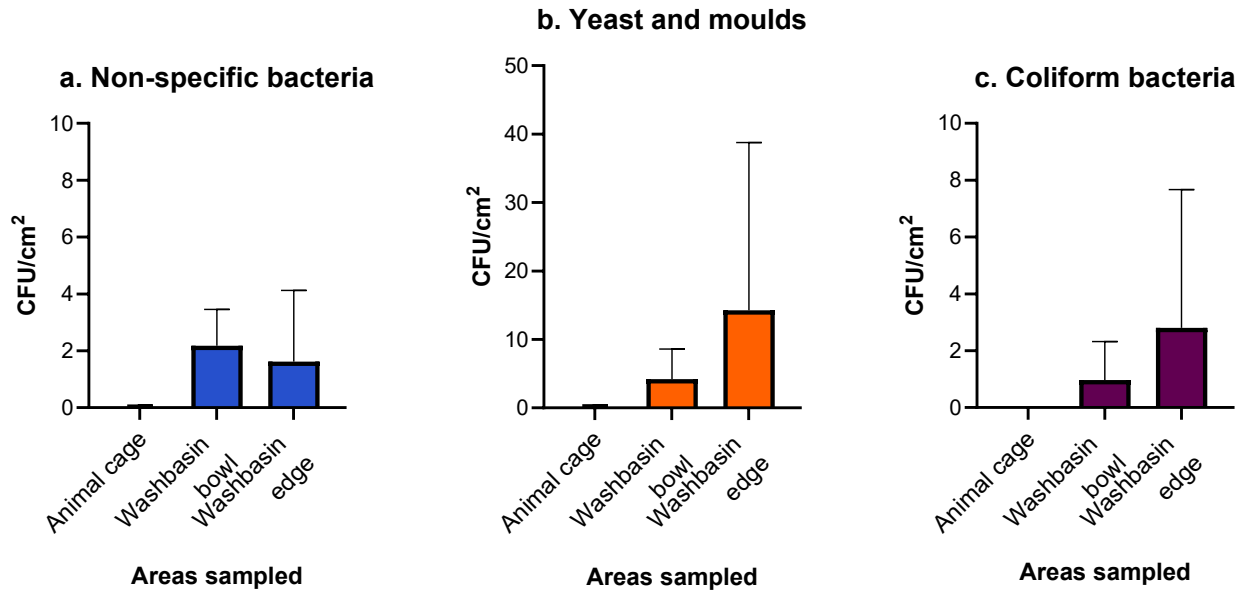


Figure 3.10: Mean CFU/cm^2 of contact plates in the washing area, on three different growth media, over three days. (a) Non-specific bacteria, (b) yeast and moulds, and (c) coliform bacteria. Bar graph indicates the mean, and error bars indicate the standard deviation.

3.4.1.3 Microbial air samples in different rooms in the facility

Microbial air samples were taken to capture bioaerosols that could be inhaled by the workers in the facility. Air samples containing microorganisms were collected on three different media over three days. Blood agar plates (fastidious bacteria) were added on the third day due to the observation of growth from the previous days and the assumption of the possible presence of fastidious bacteria. The fastidious bacteria plates in the breeding room yielded a concentration of 46 CFU/m^3 consisting of beta haemolysis growth, and the washing area yielded a concentration of 54 CFU/m^3 growth consisting of alpha and beta haemolysis growth (results not shown). The cloak room had the highest concentrations of non-specific bacteria, yeast and mould, and coliform bacteria (mean ranks = 8.1, 7.3 and 8.1, respectively). These were followed by the breeding room (mean ranks = 7.1, 6.8 and 8.0, respectively), the experimental room (mean ranks = 6.3, 6.0 and 5.3, respectively), and the washing area (mean ranks = 4.3, 5.8 and 4.5, respectively) (Figure 3.11 a, b and c). However, the Kruskal-Wallis test revealed no statistically significant differences between the sampled areas, and Dunn's multiple comparisons confirmed that all pairwise contrasts were non-significant (all $p > 0.9$). No outliers were noted in the MAS samples.

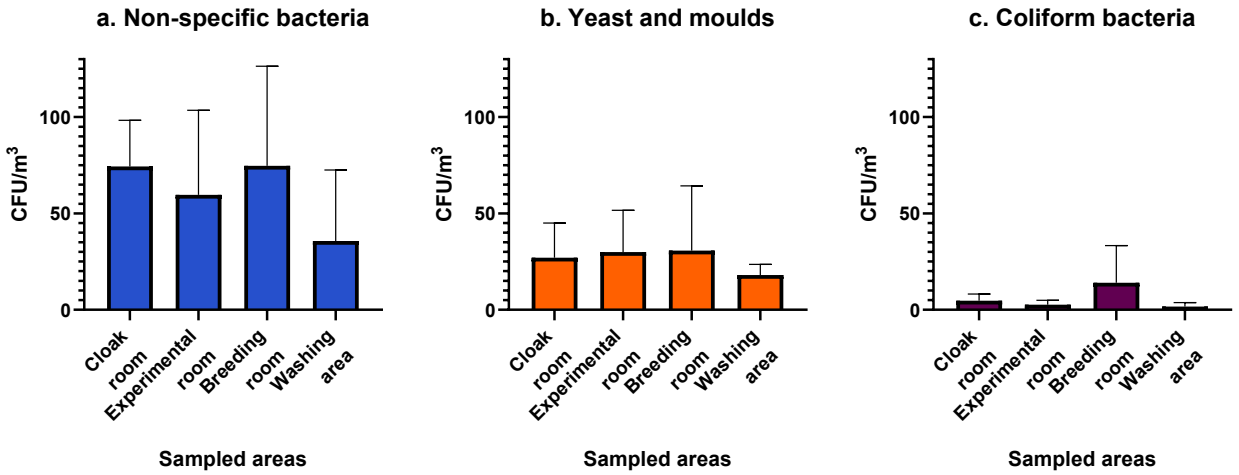


Figure 3.11: Mean CFU/m^3 of the microbial air sampler on four different sampling media, in four different sampling areas, over three days. (a) Non-specific bacteria, (b) yeast and moulds and (c) coliform bacteria. Bar graph indicates the mean, and error bars indicate the standard deviation.

3.4.1.4 Settle plates

Settle plates were used to collect airborne microbial particulates that settle on surfaces over time. An outlier of non-specific bacteria on the second day with a concentration of 14.67 CFU/cm^2/h was noted (z-score = 5.10) (results not shown). A Kruskal-Wallis test followed by Dunn's multiple comparisons produced statistically insignificant ($p > 0.2$) comparisons of non-specific bacteria, and yeast and mould media indicating that the highest concentration of microbial growth was in clean hallway 1 (mean rank = 6.6 and 5.5 respectively), then clean hallway 2 (mean rank = 5.5 and 4.8 respectively) and the dirty hallway (mean rank = 2.8 and 4.6 respectively) with the lowest concentrations (Figure 3.12 a and b). Coliform bacteria showed the highest concentrations in clean hallway 1 (mean rank = 5.6), followed by the dirty hallway (mean rank = 5.3), and the lowest in clean hallway 2 (mean rank = 4.0). These differences were statistically insignificant ($p > 0.9$) (Figure 3.12 c).

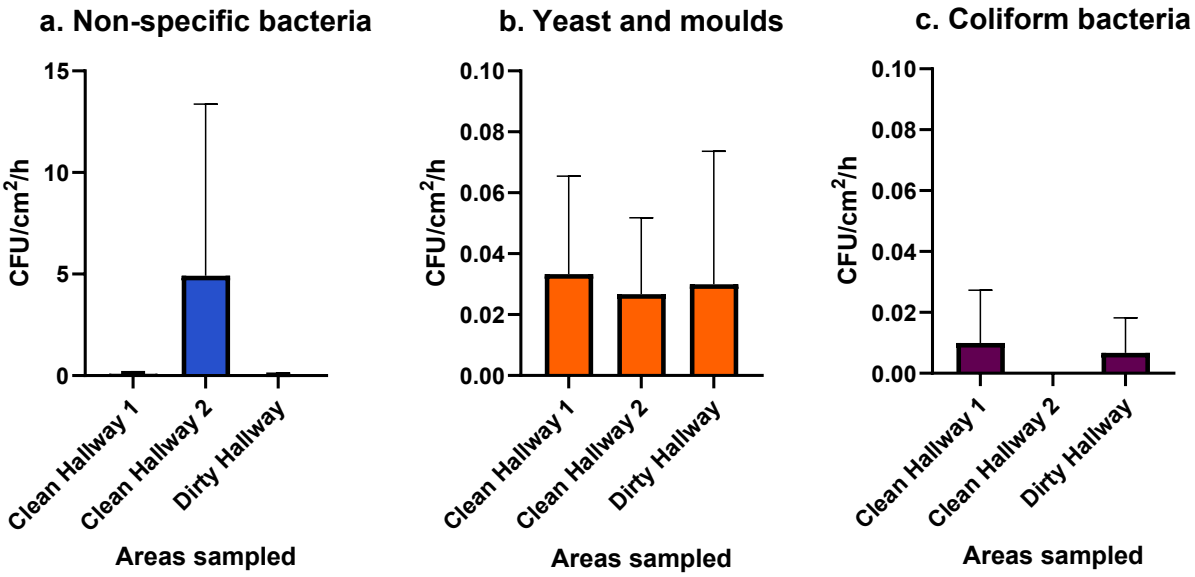


Figure 3.12: Mean CFU/cm^2/h of the settle plates in the three hallways, on three different growth media, over three days. (a) Non-specific bacteria, (b) yeast and moulds, and (c) coliform bacteria. Bar graph indicates the mean, and error bars indicate the standard deviation.

3.4.2 Metabarcoding

The samples that underwent sequencing were grouped according to sampling method, area and day.

3.4.2.1 Diversity indices

The observed index measures the number of different species detected in a sample. A higher value means more species were present (Gotelli & Colwell, 2001:379). The highest richness value (> 600) was observed for a contact plate sample collected in the breeding room (Figure 3.13). A second high value, just below 400, was recorded for a surface swab sample collected in the washing area. Most samples from the breeding room, cloak room and washing area showed moderate richness, generally between about 100 and 300. Lower richness values, including several values close to zero, were recorded across multiple methods in the hallways and experimental room.

The Shannon diversity index measures the diversity of species by combining richness and evenness of a sample; the higher the value, the higher the diversity and a value that equals zero indicates only one species in the sample (Kers & Saccenti, 2022:3–4). The highest Shannon values were recorded in surface swab samples from the washing area and in a cyclone sample from the breeding room (Figure 3.13). Several microbial air samples collected in the washing area, cloak room and breeding room also showed higher Shannon values. Moderate Shannon values were observed across most sampling methods and areas, typically between 1 and 2. Lower Shannon values, close to zero, were

also present across the dataset, occurring from all other sampling methods, except for cyclone and surface swabbing samples, and all sampling areas despite the generally higher values observed in many cases.

The Simpson's index measures how evenly species are distributed in a sample. It considers both the number of species and how dominant each one is. Lower values indicate higher diversity, while higher values show that only a few species dominate (Magurran, 2021:1175). The majority of the samples had high Simpson values, indicating a more even distribution and less dominance of a specific species (Figure 3.13). The highest Simpson values appeared in several surface swab samples collected in the washing area and in microbial air samples from the washing area, cloak room and breeding room. Cyclone samples also showed high Simpson values. Lower Simpson values were observed in individual samples from all sampled areas across all sampling methods.

The PCoA Bray–Curtis index is a method used to compare how similar or different microbial communities are. Using an index that goes from 0 (complete similarity) to 1 (complete dissimilarity), the compositional variations in species abundances among the samples were evaluated (Ramette, 2007:148). The Bray–Curtis index measures the difference in species and their abundances between samples, while PCoA displays these differences on a graph so that patterns or groupings can be easily seen (Figure 3.14). The PCoA plot showed that all samples had similar values, with Bray-Curtis distances averaging approximately 0.25. The microbial air samples grouped together on the left side of the plot, with several cyclone samples close to this grouping. Additional cyclone samples appeared more dispersed across the graph. The surface swabs, settle plates and contact plates were predominantly grouped on the right side of the graph, forming a separate cluster from the microbial samples. The surface swab and settle plate samples showed tight clustering, and most contact plate samples were positioned within the same region, with a slightly wider spread. Overall, the graph showed two main clusters, consisting of microbial air samples with some cyclone samples on one side of the plot and the surface swabs, settle plates and contact plate samples on the opposite side.

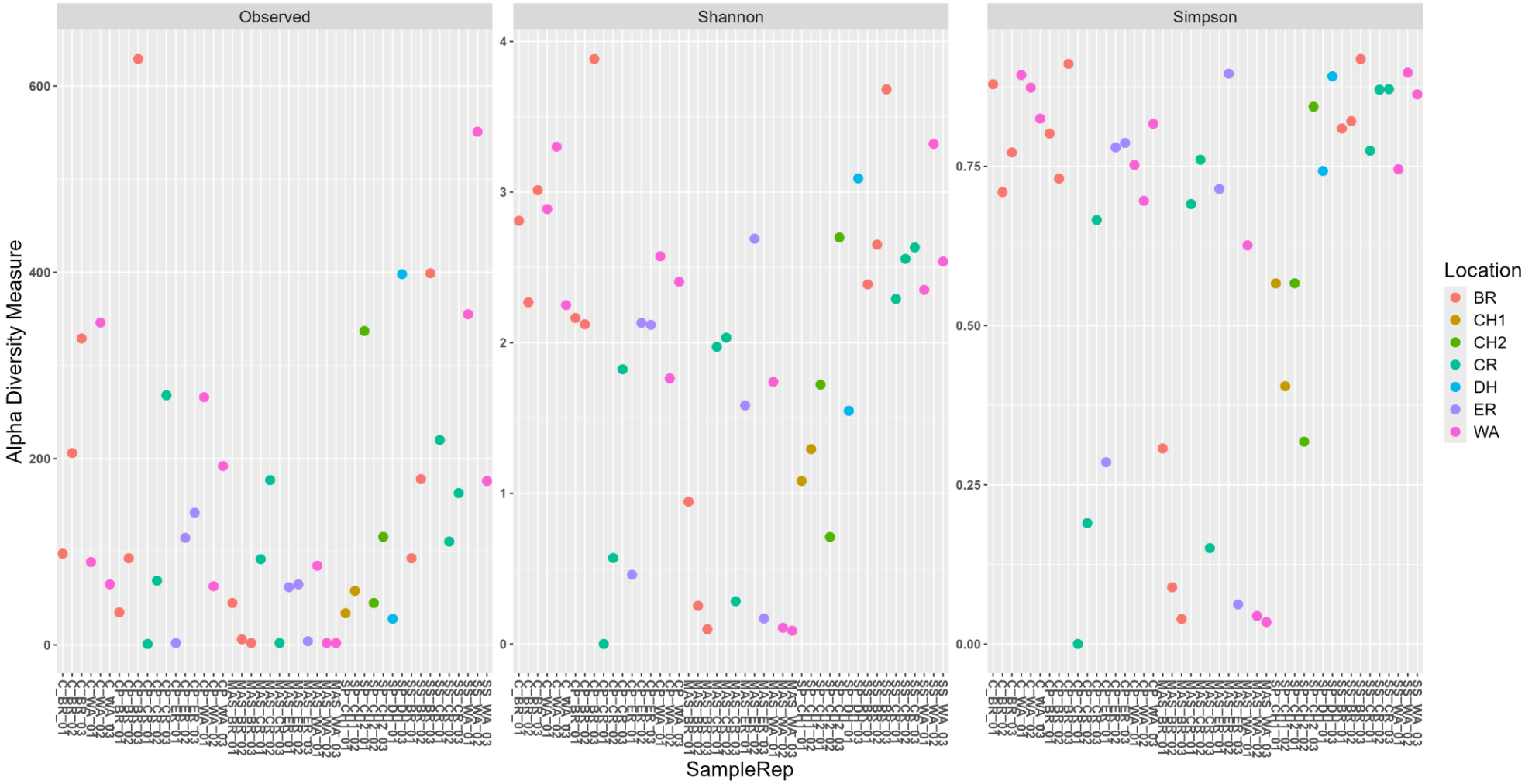


Figure 3.13: Alpha diversity expressed as observed, Shannon and Simpson indices. (CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway). The numbers at the end indicate the three sampling days/repeats.

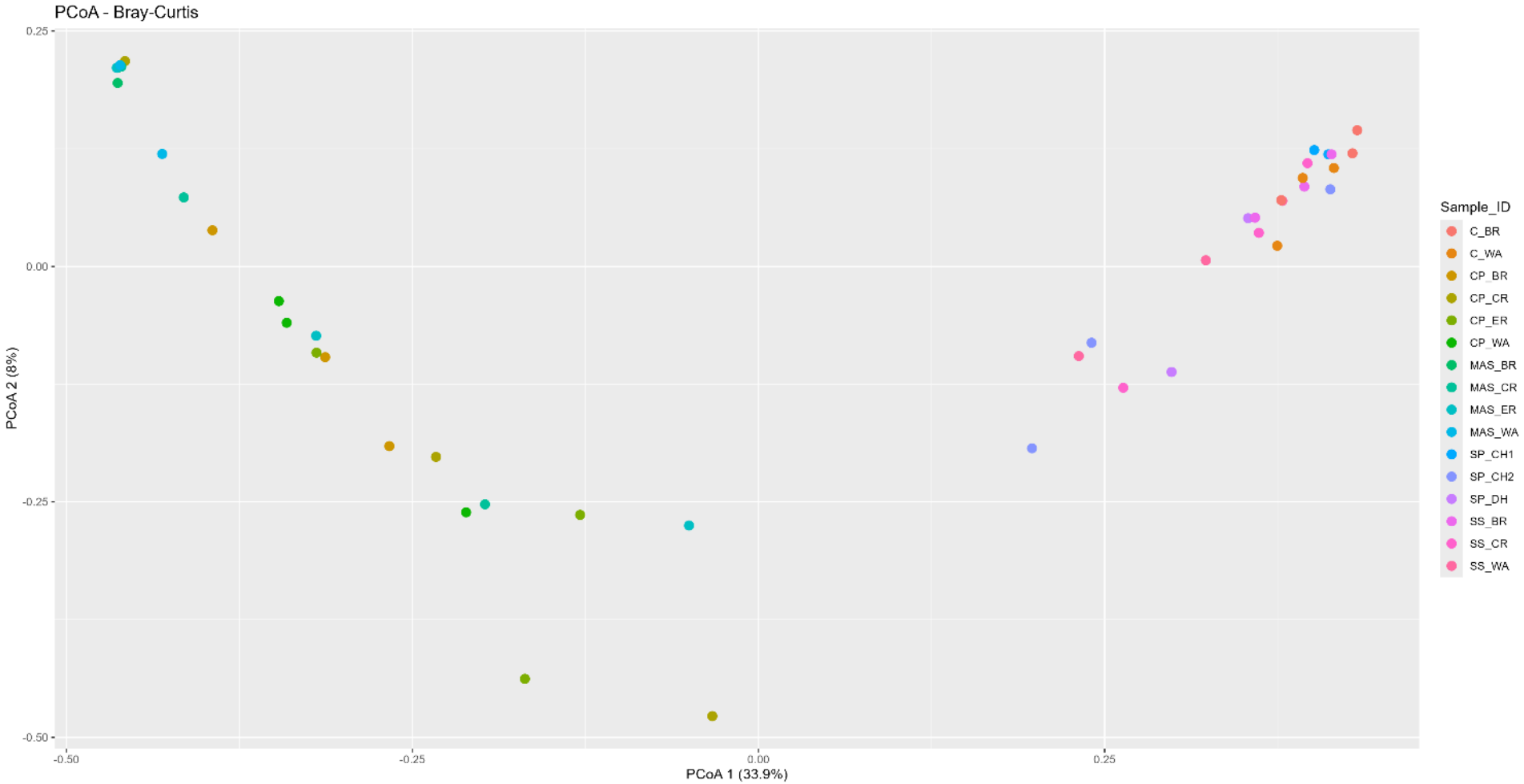


Figure 3.14: Beta diversity expressed as the PCoA-Bray-Curtis index. (CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway).

3.4.2.2 Taxonomic abundance

Of all the samples, the most dominant phyla were *Pseudomonadota* (61.3%), *Bacillota* (25.9%) and *Actinomycetota* (12.3%). Less dominant, but still notable, were *Ascomycota*, *Bacteroidota* and *Chordata*, each attributing approximately 0.1% of the total abundance. All other phyla detected had relative abundance below 0.01%. Interestingly, *Pseudomonadota* was the most abundant phylum in the microbial air samples, exceeding the levels observed in the other sampling methods (Figure 3.16).

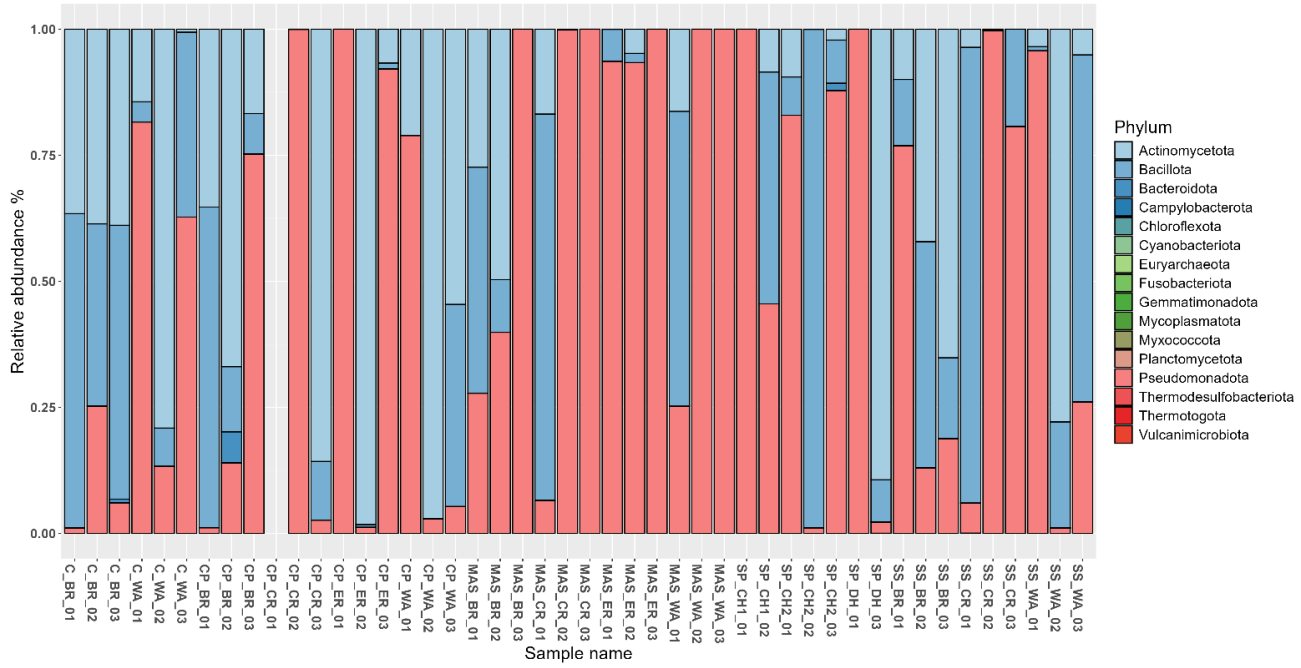


Figure 3.15: Stacked bar chart of abundance taxa at the phylum rank in all 46 samples. The y-axis indicates the relative abundance of each taxon in percentage for each sample. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

At the class level, the most abundant identified groups were *Gammaproteobacteria* (44.09%), *Actinomycetes* (29.88%) and *Bacilli* (20.72%), which together represented the majority of classified sequences across all samples once unassigned reads were removed. Lower-abundance classes included *Alphaproteobacteria* (3.14%) and *Betaproteobacteria* (1.81%), with several minor contributors such as *Mammalia* (0.07%), *Sphingobacteriia* (0.06%), *Eurotiomycetes* (0.04%), *Sordariomycetes* (0.02%) and *Flavobacteriia* (0.02%). All remaining classes were present at below 0.01% relative abundance (Figure 3.17).

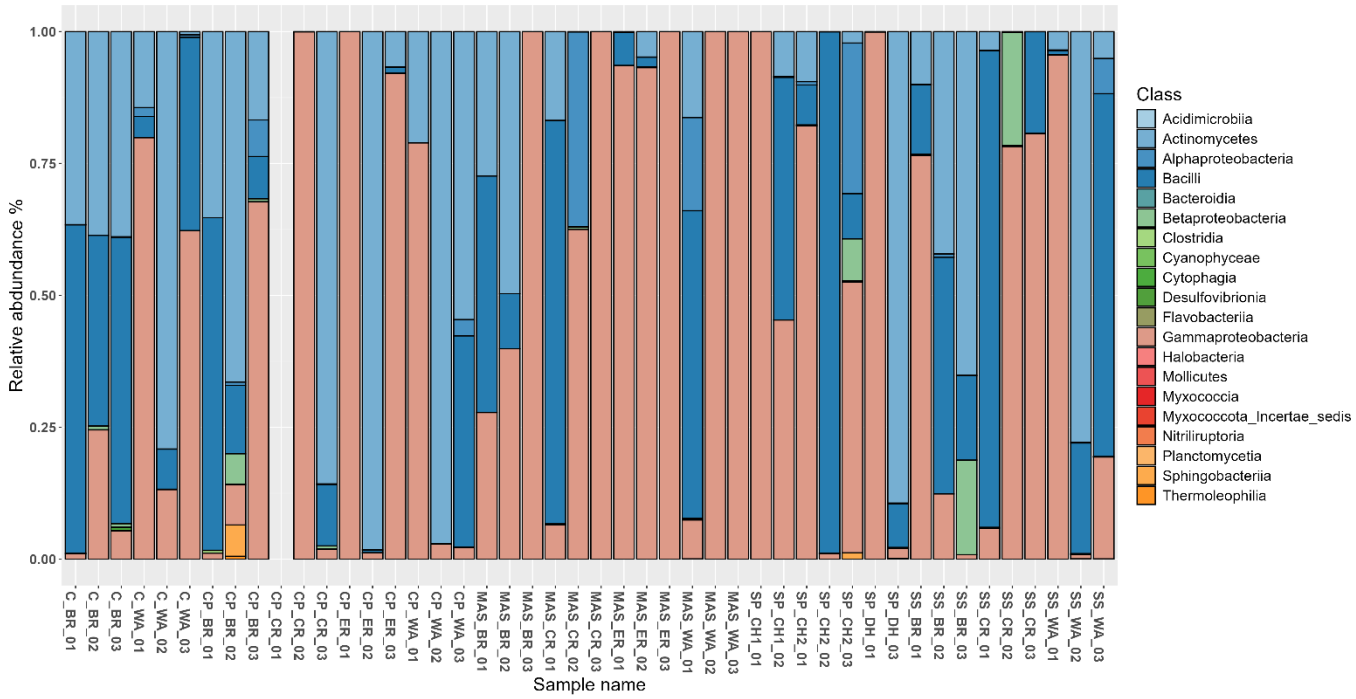


Figure 3.16: Stacked bar chart of abundance taxa at the class rank in all 46 samples. The y-axis indicates the relative abundance of each taxon in percentage for each sample. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

At the order level, the most abundant groups were *Micrococcales* (27.12%), *Bacillales* (20.56%) and *Pseudomonadales* (19.30%), forming the dominant portion of the identified community. Additional orders appearing at moderate abundance included *Corynebacteriales* (7.89%), *Actinomycetales* (6.05%) and *Enterobacterales* (2.48%). Lower-abundance contributors included *Mycobacteriales* (2.36%), *Burkholderiales* (1.79%) and *Vibrionales* (0.73%), while all remaining orders occurred only at trace levels (Figure 3.18).

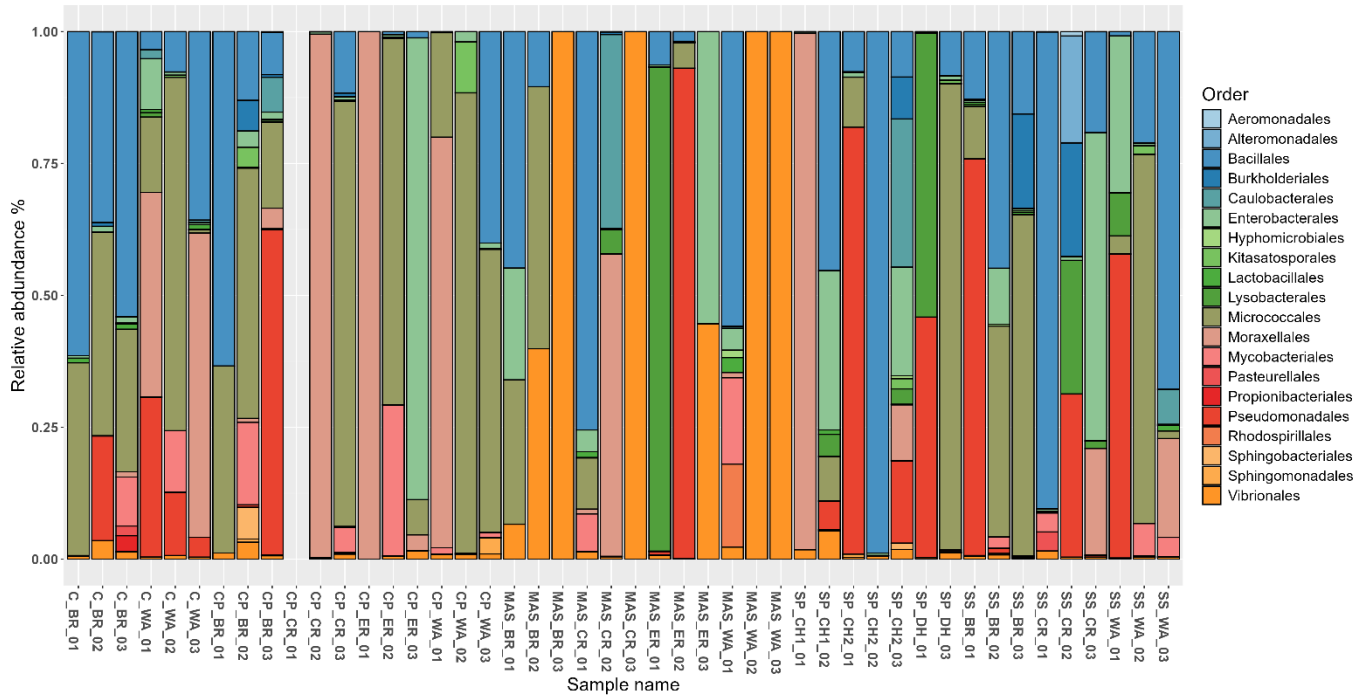


Figure 3.17: Stacked bar chart of abundance taxa at the order rank in all 46 samples. The y-axis indicates the relative abundance of each taxon in percentage for each sample. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

At the family level, the most abundant groups were *Microbacteriaceae* (21.23%), *Pseudomonadaceae* (20.46%), *Moraxellaceae* (13.72%) and *Micrococcaceae* (9.84%), which together accounted for the majority of identified families across the combined dataset. Other families were present at lower proportions, including *Bacillaceae* (5.72%), *Paenibacillaceae* (3.76%), *Staphylococcaceae* (3.07%), *Nocardiaceae* (2.12%), *Intrasporangiaceae* (1.72%) and *Xanthomonadaceae* (1.41%). All remaining families contributed less than 1% to the total identified community (Figure 3.19).

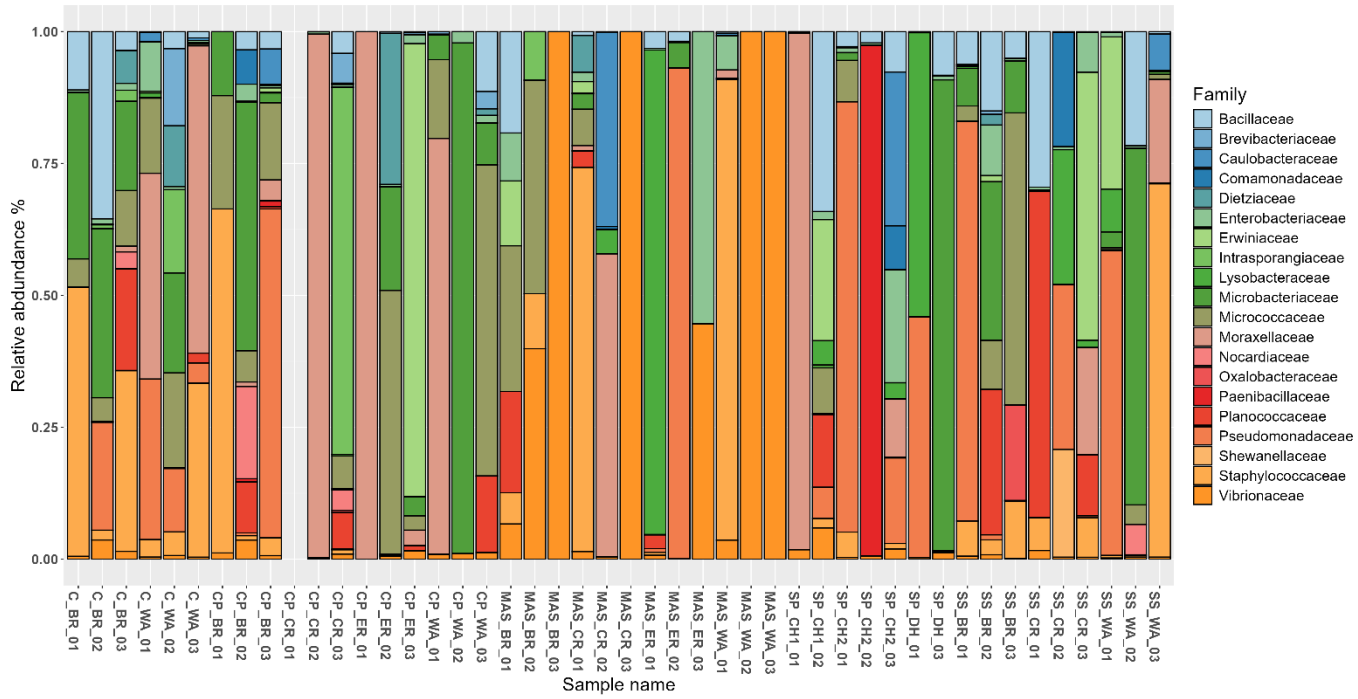


Figure 3.18: Stacked bar chart of abundance taxa at the family rank in all 46 samples. The y-axis indicates the relative abundance of each taxon in percentage for each sample. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

At the genus level, the most abundant identified organisms were *Acinetobacter* (13.02%), *Stutzerimonas* (10.62%), *Curtobacterium* (9.04%) and *Pseudomonas* (8.99%), forming the core of the resolved microbial community structure. Additional genera detected at moderate to low abundance included *Janibacter* (5.55%), *Arthrobacter* (4.84%), *Microbacterium* (4.60%), *Pantoea* (3.70%), *Sporosarcina* (2.55%), *Paenibacillus* (2.28%), *Kocuria* (1.83%) and *Shewanella* (1.68%). All other genera occurred at less than 1% relative abundance (Figure 3.20).

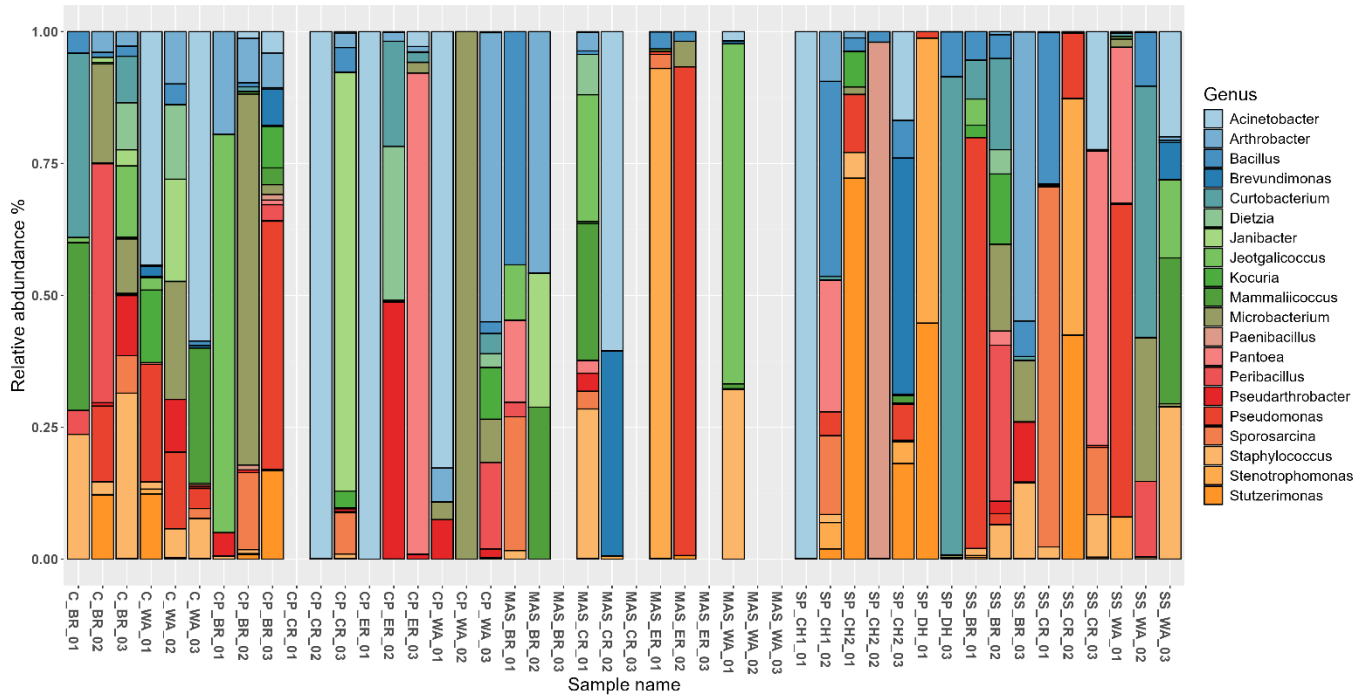


Figure 3.19: Stacked bar chart of abundance taxa at the genus rank in all 46 samples. The y-axis indicates the relative abundance of each taxon in percentage for each sample. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

At the species level, the most abundant identified organisms were *Stutzerimonas stutzeri* (9.83%), *Paenibacillus thiaminolyticus* (4.35%), *Acinetobacter johnsonii* (3.97%), *Acinetobacter lwoffii* (3.71%), *Janibacter melonis* (3.24%), *Pseudomonas moraviensis* (2.74%), *Acinetobacter schindleri* (2.63%), *Pantoea vagans* (2.16%), *Mammaliicoccus lentus* (1.92%) and *Brevundimonas diminuta* (1.64%). All remaining identified species occurred at less than 1% relative abundance (Figure 3.21).

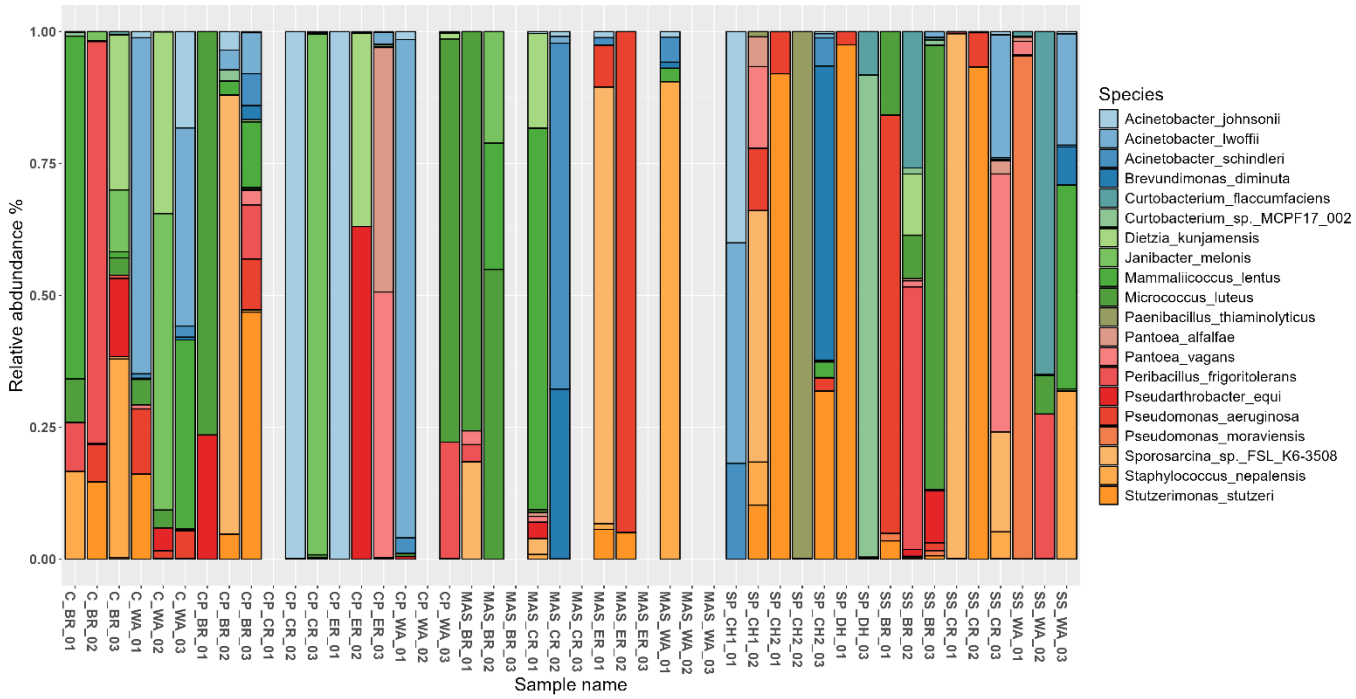


Figure 3.20: Stacked bar chart of abundance taxa at the species rank in all 46 samples. The y-axis indicates the relative abundance of each taxon in percentage for each sample. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

3.4.2.3 Identification based on Nanopore sequencing results

A total of 59 risk group 2 HBAs were identified in the facility, 53 of them bacterial and 6 of them fungal. Three risk group 3 HBAs were also identified in the facility and a total of five agents identified are toxin producing HBAs (Table 3.2).

Table 3.2: Genus and species identified using nanopore sequencing within the animal testing facility and categorised into the risk groups outlined in the Hazardous Biological Agents Regulations of South Africa, 2022 (Department of Employment and Labour, 2022:24–30)

Biological agent	Notation	Biological agent	Notation
Risk group 2 bacteria, rickettsiae and mycoplasmas			
Achromobacter spp.		*Klebsiella spp.*	
Acinetobacter spp.		*Lactobacillus spp.*	
Actinomadura spp.		*Leclercia adecarboxylata*	
Actinomyces spp.		*Legionella spp.*	
Aeromonas spp.		*Listeria spp.*	
Arcanobacterium spp.		*Moraxella spp.*	
Arthrobacter spp.		*Nocardia spp.*	
Bacillus cereus		*Photobacterium spp.*	
Bordetella spp.		*Propionibacterium spp.*	
Brevibacterium spp.		*Proteus spp.*	
Brevundimonas diminuta		*Providencia spp.*	
Burkholderia spp.		*Pseudomonas spp.*	
Chryseobacterium spp.		*Rhodococcus spp.*	
Citrobacter spp.		*Salmonella spp.*	
Clavibacter michiganesis		*Serratia spp.*	
Clostridium spp.		*Shigella spp.*	
Clostridium tetani	T, V	*Sphingobacterium spp.*	
Corynebacterium spp.		*Sphingomonas spp.*	
Curtobacetrium flaccumfaciens		*Staphylococcus spp.*	
Edwardsiella spp.		*Staphylococcus aureus*	T
Enterobacter spp.		*Stenotrophomonas spp.*	
Enterococcus spp.		*Streptococcus spp.*	
Erwinia spp.		*Tsukamurella spp.*	
Escherichia spp.		*Ureaplasma spp.*	
Gemelli spp.		*Vibrio spp.*	
Gordonia spp.		*Vibrio cholera*	T, V
Haemophilus spp.			
Risk group 2 fungal agents			
Absidia spp.		*Exophiala spp.*	
Aspergillus spp.		*Fusarium spp.*	
Cladophialophora spp.		*Rhizopus spp.*	
Risk group 3 bacteria, rickettsiae and mycoplasmas			
Bacillus anthracis	V	*Shigella dysenteriae*	T
Escherichia coli	T		

Notations: T – toxin production and V – vaccine available.

From all 46 samples, majority of the samples contained *Homo sapiens* DNA (Figure 3.22). A total of 10 samples did not contain *Homo sapiens* DNA, six of which were obtained from the cyclone sampler. The other culture-dependant samples that did not contain *Homo sapiens* DNA were two from the settle plates, one in clean hallway 1 on the second day and the other one in the dirty hallway on the first day, one from the surface swabs in the breeding room on the third day and the last one from the contact plates in the washing area on the second day.

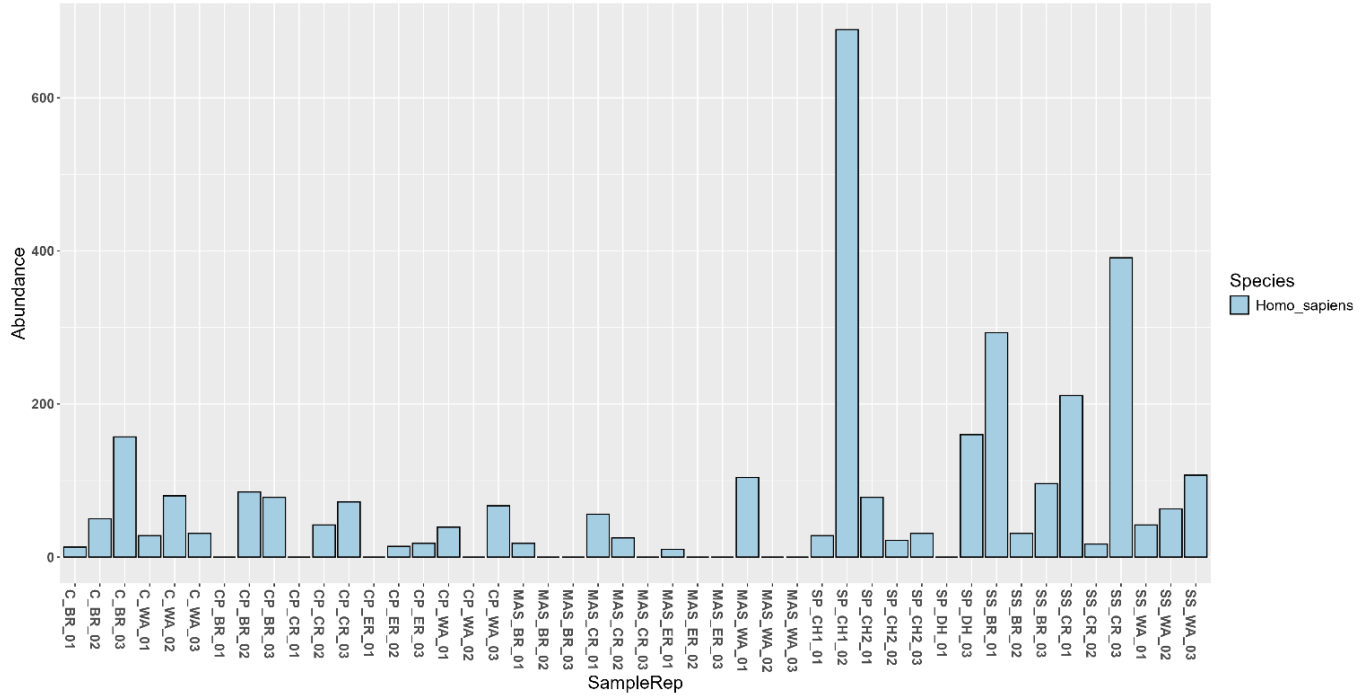


Figure 3.21: Figure of Eucaryotic abundance, specifically of the species *Homo sapiens* of each of the 46 samples. The following abbreviations are used for the sampling methods and areas: CP – contact plates; MAS – microbial air samples; SP – settle plates; SS – surface swabbing, C – cyclone; CR – cloak room; ER – experimental room; BR – breeding room; WA – washing area; CH1 – clean hallway 1; CH2 – clean hallway 2 and DH – dirty hallway. The numbers at the end of the x-axis label indicate the three sampling days.

Settle plates from the hallways collected the DNA of two viruses. *Arthrobacter* phage Bauer from clean hallway 1 on the second day and *Arthrobacter* phage Nandita from the dirty hallway on the third day.

3.4.3 Physical environmental parameters within the animal testing facility

The temperatures within the different sampling areas ranged closely between 18 ± 2.4°C and 21 ± 1.4°C (Table 3.2). The RH ranged between 27.7 ± 12.3% and 37.0 ± 16.8%, with the highest percentages in the cloak room and washing area and the lowest percentages in the dirty hallway. The CO_2 concentrations ranged between 394 ± 52 ppm and 777 ± 203 ppm. The cloak room and

experimental room yielded the highest CO_2 concentrations whereas the dirty hallway yielded the lowest. The CO ranged from 0.0 ± 0.1 ppm to 0.4 ± 0.5 ppm with the lowest concentration in the cloak room and experimental room and the highest in the dirty hallway. The air velocity ranged from 0.02 ± 0.02 m/s in the cloak room to 0.07 ± 0.04 m/s in clean hallway 1 (Table 3.2).

Table 3.3: Mean and standard deviations of the physical parameters for each sampling location.

	Temperature (°C)	Relative Humidity (%)	CO_2 concentration (ppm)	CO concentration (ppm)	Air velocity (m/s)
Cloak room	18 ± 2.4	37.5 ± 11.8	777 ± 203	0.0 ± 0.1	0.02 ± 0.02
Experimental room	19.8 ± 0.4	31.8 ± 14.1	777 ± 203	0.0 ± 0.1	0.03 ± 0.01
Breeding room	20.3 ± 1.4	32.0 ± 12.2	611 ± 95	0.3 ± 0.4	0.05 ± 0.03
Washing area	21.5 ± 1.9	37.0 ± 16.8	464 ± 35	0.1 ± 0.1	0.06 ± 0.04
Clean hallway 1	20.2 ± 1.0	29.6 ± 13.3	448 ± 18	0.2 ± 0.3	0.07 ± 0.04
Clean hallway 2	20.5 ± 0.9	28.8 ± 13.3	473 ± 28	0.3 ± 0.3	0.06 ± 0.06
Dirty hallway	21 ± 1.4	27.7 ± 12.3	394 ± 52	0.4 ± 0.5	0.03 ± 0.03

ppm = parts per million. m/s = meters per second

From all Spearman correlation coefficients that were calculated, many showed to have strong correlations; however, none of the correlations calculated showed any statistical significance. The table containing all Spearman correlation coefficients can be found under supplementary material (Table S1).

3.4.4 Dust particulate matter within the animal testing facility

All sampled areas over all three days yielded average concentrations lower than 0.001 mg/m^3 except for the washing area on the third day, which yielded a concentration of 0.0036 ± 0.0044 mg/m^3. Correlations were done between the concentrations of dust particulate matter (smaller than 4 µm) and the airborne microbial concentrations (CFU/m^3) of each room. Correlations for the experimental room, breeding room and washing area were all the same ($r = -0.5$, $p > 0.9$), found to be weak and statistically insignificant. A strong negative correlation was found between the airborne microbial growth and the dust particulate matter in the cloak room; however, it was found to not be statistically significant ($r = -1.0$, $p = 0.3$).

Figure 3.23 a, b and c illustrate the dust particulate matter concentration of specific particulate size fractions ranging from 0.3 to 2.793 µm in the unit of "1000 counts/m^3", meaning that each value

represents how many thousands of particles were detected in one cubic meter of air. For example, a reading of three 1000 counts/m^3 is equal to 3000 particles per cubic metre. The washing area consistently yielded the highest concentrations of dust particulate matter of all the size fractions, with the smallest size fractions (< 1 µm). The larger the size fraction, the lower the concentration (Figure 3.23 a). The breeding room on the third day yielded the lowest concentrations of dust for all size fractions of the three days.

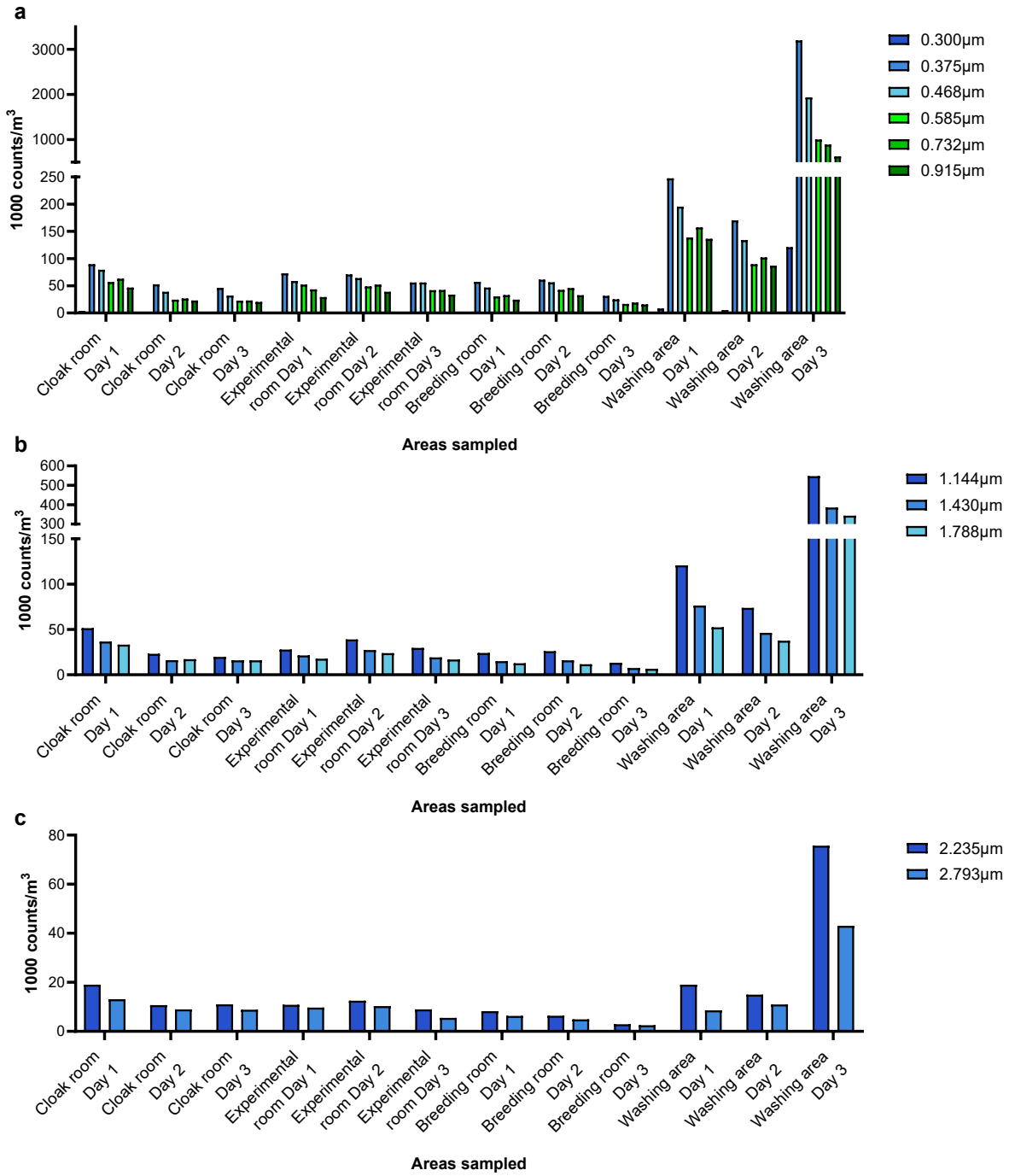


Figure 3.22: Dust particulate matter in size fractions. Size fractions which fall under the respirable fraction, specifically summarised in (a) particles smaller than 1 µm, (b) particles between 1 µm and 2 µm, and (c) particles between 2 µm and 3 µm.

3.5 Discussion

This study aimed to determine the presence of hazardous biological agents within an animal testing facility, and furthermore to identify these agents. Specifically, to determine to what extent the HBAs found in an animal testing facility are hazardous to the workers' health, as categorised into the risk groups according to the South African Hazardous Biological Agents Regulations, 2022 (Department of Employment and Labour, 2022:23–33). This determination was done by assessing all aspects of the discussion and anticipating the extent to which the HBAs found in the facility are hazardous.

3.5.1 Microbial growth in the facility

The cloak room provided high concentrations of growth throughout all sampling methods, the growth of the surface swabs from both the clean and dirty floors provided the highest concentrations. This is important to note due to the cloak room being the area where PPE is donned and doffed by the workers when entering and exiting the facility. Workers can unknowingly be exposed to HBAs due to contamination in this area, which is supposed to be a clean area, and indicates that this area could contribute to contamination entering the facility. This could be because workers possibly step on dirty bedding or other contaminated sources in the dirty area and then they transport the HBAs via the shoe covers when exiting the facility and contaminate the clean part of the facility. Contact plate growth from the clean lab coats could indicate that contamination might enter the facility via the lab coats. The air samples also yielded the highest concentrations compared to the rest of the facility, indicating that the increase of microorganisms in this area could be due to the small size of the room, lack of connectivity to the rest of the facility's ventilation system as well as the HBAs increasing due to the workers entering the room to don the PPE (Lipman & Leary, 2015:1588–1589).

Active experimental rooms were inaccessible due to the risk of additional people in the rooms adding stress, may negatively affect the animal test subjects. This fact resulted in an inactive experimental room being used as a baseline indicator for exposure. The experimental room samples yielded the third-highest airborne microbial concentrations. On the first day of sampling, the animal cage ventilation system was still functioning in the room with no cages attached, but with the next sampling days the entire ventilation system was removed. This fact could lead to speculation that the contamination in the room could have come from recent active experiments in the room as well as from the workers when they entered and exited the room while removing the ventilation system to be cleaned and stored until needed for the next use (Hospodsky *et al*., 2012:11). The air supply vent provided low to no concentrations of microbial growth, as found across other sampled areas of the facility. This suggests that the air provided to the facility does not serve as a source of exposure and that the HEPA filters used by the facility are effective at removing or preventing contaminated air from entering the facility through the centralised ventilation system (Lipman & Leary, 2015:1588–1589). A

high concentration of growth was found on the contact plate samples from the extraction ventilation grate and could indicate that the majority of contamination comes from the workers and animals.

The breeding room yielded the second highest airborne microbial concentrations on average, identifying this area as a high-risk area. This indicates the need for stricter control measures to be implemented, such as the use of FFP2 respirators instead of wearing surgical masks not providing an airtight seal to protect against inhalation of bioaerosols (Coffey *et al*., 2020). The high concentrations in the breeding room could be due to the high number of animal cages (approximately 280 cages) and animal cage occupancy (approximately five animals per cage). The animal cages are connected to an independent ventilation system, which ensures that the air from the cages does not escape into the room (Lipman & Leary, 2015:1548). However, the animals are frequently handled for purposes such as bedding and cage exchanges and examination of the animals when the cages are opened. This could potentially lead to air and particulates from the cages being emitted into the room (Fong, 2017:15). The surface swabs and contact plates of the breeding room had the highest concentrations of growth at the washbasins and workstations, which is expected, as these are the most commonly used surfaces in the room.

Hospodsky *et al*. (2012:1) investigated the influence of human occupancy on aerosol mass and bacterial genome concentration in university classrooms. They found that occupancy of the classrooms increased the aerosol mass and bacterial genome concentration by nearly two orders of magnitude in airborne bacterial genome concentration in PM_{10}. They concluded that the increase in activity in the room resuspended dust containing microbial particulates, which increased the concentration of microbes in the air. The low concentrations in the hallways of the facility in this study could be due to low activity that was observed during the sampling period, supporting Hospodsky *et al.*'s (2012:1) findings.

The washing area, although in the dirty part of the facility, yielded the lowest concentration of air microbial growth. This could be because the animal bedding has already been removed from the cages before the cages are moved to this area for cleaning and sanitation, working mainly with empty dirty cages. However, despite this procedure being in place, dirty animal bedding was still observed in the biohazardous waste disposal bin during the sampling period, which could have accounted for the growth found. The surface swabbing and contact plates in the washing area had the highest concentration of growth on the washbasins and workstations, which is expected as these areas are the most used surfaces within the area.

Kaliste *et al*. (2004:25) assessed the used bedding in animal testing facilities for bacterial, fungal and endotoxin contamination by capturing airborne particulate matter produced in a controlled manner on an MCE filter fitted with a sampling pump. They concluded that the dirty bedding may contain

biologically effective compounds possibly distributed into the ambient air. Mayeux *et al*. (1995:2297) conducted studies in American animal testing facilities to test for contamination of clean bedding before use by the animals. They used a combination of settle plates and an Anderson air sampler, and they found that most of the bedding that was not heat-treated had contamination of fungal spores directly from the manufacturer. They highlighted the importance of examining for microbial contamination before use. Even though a different sampling method was used in this study compared to those of Kaliste *et al*. (2004:25) and Mayeux *et al*. (1995:2297), results obtained from this study can still be compared. The samples of the clean bedding yielding no growth conflict with the results obtained from Mayeux *et al*. (1995:2297) and the positive growth of the dirty animal bedding coincides with that of Kaliste *et al*.'s (2004:25) findings, however, the extent to which the dirty bedding is hazardous cannot be determined without further analysis, such as nanopore sequencing.

3.5.2 Metabarcoding

3.5.2.1 Diversity

The washing area was identified to be the area with the highest diversity out of all the samples. This is an expected result as this area is not a controlled environment like the rest of the facility. The surface swabbing samples in general provided the highest alpha diversity for the observed, Shannon and Simpson diversity indices, suggesting that the air contained a broader range of microbial taxa when compared to the microbial air sampler and contact plate samples. This coincides with the opinions of Viegas *et al*. (2020:9) and Garcia-Alcega *et al*. (2017:75). Viegas *et al*. (2020:9) states that passive sampling methods provide a wider diversity than that of active sampling methods and Garcia-Alcega *et al*. (2017:75) also states that the active impaction sampling method could reduce the retrieval of culturable microorganisms, further stating that a large variety of microbes are excluded when choosing a culture dependent method because less than 1% of viable microbes are culturable. The microbial air sampler did however provide the second highest diversity out of all the sampling methods. The settle plates had the lowest diversity from the sampling methods. There could be two reasons to explain this. The first reason is that the settle plates were left out for approximately 1.5 hours, not long enough for the smaller particles to settle onto the plates. This was due to the limited time the researcher had access to the facility and left the plates out for the maximum amount of time that was offered. Eaton (2022:2) explains that a 4-hour period is recommended to give the smaller particles a chance to settle and to keep the agar from drying out being exposed to air too long. The other reason could be due to the placement of the settle plates in the hallway. It was already established that the hallways had overall the lowest concentration of microbial growth, and the only other samples from the hallways were those of the air supply ventilation, whose abundance was so low that they were not selected for sequencing. The cyclone sampler had the second lowest diversity of all the samples. This could be due to the sampling time being too short and a lack of microbial

agents to collect during sampling. Although NIOH does not state a sampling time, studies of the cyclone sampler's efficiency (Cao *et al*., 2011:1; Noti *et al*., 2012:1569) have used periods of 60 minutes each in controlled environments. However, another study conducted by Verreault *et al*. (2011:492) in a cheese factory sampled the entire process, which was approximately 12 hours or 7200 minutes. When taking Verreault *et al*.'s (2011:492) study into account, the conclusion can be made that standard occupational hygiene sampling principles should be applied to collect samples during the entire working shift (8 hours) to get a representative sample. None of these procedures were followed in this study due to a limited time of access to the facility, leading to the cyclone only being able to run for 45 minutes in each room.

The beta diversity results demonstrated that the microbial communities detected across the facility were largely similar, as reflected by the low Bray–Curtis dissimilarity values. However, the microbial air samples grouped closely together, suggesting that the microbial air sampler method consistently captured comparable airborne microbial communities across sampling days. Some cyclone samples also grouped near the microbial air sampler cluster, although the wider spread of the cyclone samples indicates that this method collected a more variable community profile. In contrast, the surface swabs, settle plates and contact plates formed a compact cluster on the opposite side of the plot. The tight grouping of the surface swab and settle plate samples suggests that these methods detected highly similar microbial communities. The contact plate samples showed slightly more variation but still aligned predominantly with the other passive sampling methods.

Overall, these results reinforce the importance of microbial air sampling and suggest that multiple collection methods are necessary to fully capture the extent of microbial exposure risk. This is because the microbial air samples provide a good estimate as to how many microbes are in the room, the settle plates indicate the particles that were airborne and have settled onto surfaces, leading to surface contamination. The cyclone sampler can provide more detail about the microbial particulate sizes, specifically particles in the respirable fraction (estimated ≤ 4 µm), which can penetrate the gas exchange region of the lungs (Brown, 2013:1), as well as the ability of collecting and identifying culture independent microbes which are missed when only using culture dependant methods. Both the surface swabs and contact plates provide information about microbial contamination on both flat and irregular surfaces.

3.5.2.2 Taxonomic abundance

Across rooms and methods, the predominance of *Pseudomonadota, Bacillota* and *Actinomycetota*, with lower but notable contributions from *Ascomycota*, *Bacteroidota* and *Chordata*, is consistent with indoor/air microbiome literature. *Proteobacteria/Pseudomonadota*, *Firmicutes/Bacillota* and *Actinobacteria/Actinomycetota* typically dominate settled dust and airborne fractions in built

environments, while *Ascomycota* is the leading fungal phylum (Franchitti *et al.*, 2022:1; Gangneux *et al.*, 2020:1; Gilbert & Hartmann, 2024:742). These groups reflect the mixed origin of indoor bioaerosols, human shedding and resuspension, exchange with outdoor air, and process-specific sources such as water aerosols and surface wet work, which is exactly the source mix present in this facility (Franchitti *et al.*, 2022; Gilbert & Hartmann, 2024).

Within *Pseudomonadota*, classes and orders such as *Gammaproteobacteria* (for example, *Pseudomonadales* and *Enterobacterales*) are frequently reported in air and dust and include environmental genera associated with water and surfaces (for example, *Acinetobacter*, *Pseudomonas*) as well as occasional opportunists relevant to hygiene programmes (Gilbert & Hartmann, 2024:742; Reska *et al.*, 2024:1; Shin *et al.*, 2015:496). In the context of this study, their presence is most plausibly explained by a combination of outdoor air ingress, activity-related resuspension and water-related handling in the washing area, rather than necessarily indicating clinical contamination, this aligns with air-metagenomics work showing stable, site-specific urban air communities in which these taxa are commonly found (Franchitti *et al.*, 2022:1; Reska *et al.*, 2024:1).

Bacillota signatures, especially *Bacillales* (for example, *Staphylococcus*, *Paenibacillus*, *Sporosarcina*), are typical of indoor environments where human occupancy and movement release and resuspend skin- and dust-associated particles, the “personal microbial cloud” phenomenon shows that individuals emit ≥10^6 biological particles per hour into surrounding air (Davey *et al.*, 2016:209; Meadow *et al.*, 2015:1), which then settle on work surfaces and PPE (Franchitti *et al.*, 2022:1; Meadow *et al.*, 2015:1). In animal testing facilities, these taxa can therefore serve as practical indicators of occupancy and workflow (Franchitti *et al.*, 2022:1; Gilbert & Hartmann, 2024:1).

Actinomycetota (for example, *Micrococcales* such as *Micrococcus*, *Kocuria* and *Curtobacterium*) commonly derive from human skin, textiles and settled dust, often increasing with human occupancy and surface contact (Gangneux *et al.*, 2020:1; Gilbert & Hartmann, 2024:742; Hazarika & Thakur, 2020:443). Their co-occurrence with *Bacillota* across our highly touched areas and the cloak room is therefore consistent with expected indoor sources and with the observed activity patterns in this facility (Gangneux *et al.*, 2020:1; Gilbert & Hartmann, 2024:1).

For fungi, *Ascomycota* dominated the limited reads detected, which is the usual indoor pattern and generally reflects outdoor input plus growth on dust-borne substrates rather than sustained indoor amplification under low relative humidity (Franchitti *et al.*, 2022:1; Gangneux *et al.*, 2020:1; Wijayawardene *et al.*, 2020:1065). Given this facility’s environmental controls and the overall low fungal counts, these reads most plausibly indicate background influx and handling-related resuspension, not active colonisation. This matches air- and dust-based surveys where *Ascomycota*

is omnipresent but often at low absolute abundance in mechanically ventilated spaces (Franchitti *et al.*, 2022:1; Gilbert & Hartmann, 2024:1).

Low-level *Bacteroidota* is also unsurprising, indoor and urban air studies frequently detect gut-associated *Bacteroidota* as low-abundance transients arising from human shedding and outdoor contributions (Franchitti *et al.*, 2022:1; Gilbert & Hartmann, 2024:1). In risk terms this is best read as an indicator of background human influence rather than a direct hazard signal unless coupled with targeted pathogen detection or elevated loads (Franchitti *et al.*, 2022:1; Gilbert & Hartmann, 2024:1).

Finally, the detection of *Chordata* reads exclusively as *Mammalia*/*Primates*/*Hominidae*/*Homo* and classified to *Homo sapiens,* is consistent with known shedding of human DNA and human-associated microbes into indoor air and dust during occupancy (Gilbert & Hartmann, 2024:742; Mallatt, 2009:1; Meadow *et al*., 2015:1). In metagenomic air and dust data sets, human signatures co-occur with skin- and respiratory-associated bacteria and increase with activity, their presence here therefore corroborates the occupancy-linked interpretation of the bacterial profile (Meadow *et al.*, 2015:1; Reska *et al.*, 2024:1).

Community-level sequencing studies of indoor air settled dust and built environments consistently report *Proteobacteria* (*Pseudomonadota*), *Firmicutes* (*Bacillota*) and *Actinobacteria* (*Actinomycetota*) as the dominant bacterial phyla, with *Ascomycota* as the most prevalent fungal phylum and *Bacteroidota* typically present at lower relative abundance (Franchitti *et al.*, 2022:1; Gangneux *et al.*, 2020:1; Gilbert & Hartmann, 2024:742). These patterns have been observed across homes, offices, hospitals and urban environments and are widely interpreted as reflecting a combination of human occupancy, resuspension of dust, outdoor air exchange and contact with water and surfaces rather than site-specific contamination events (Franchitti *et al.*, 2022:1; Gilbert & Hartmann, 2024:742). At lower taxonomic levels, indoor studies frequently identify classes such as *Gammaproteobacteria*, orders including *Bacillales* and *Micrococcales*, and associated genera that encompass both environmental taxa and human-associated organisms, reinforcing the view that indoor microbiomes represent a mixed signal from occupants and the surrounding environment (Gangneux *et al.*, 2020:1; Meadow *et al.*, 2015:1). The detection of human DNA and human-associated microbial signatures in indoor sequencing datasets is also well documented and is attributed to continuous shedding of skin cells, respiratory aerosols and resuspended particles, a phenomenon described as the “personal microbial cloud” (Meadow *et al.*, 2015:1; Gilbert & Hartmann, 2024:1). Overall, the taxonomic structure observed in this study aligns closely with the dominant indoor and air microbiome profiles reported in literature, suggesting that the community composition reflects expected indoor ecological processes rather than atypical microbial enrichment.

In contrast to the extensive literature on homes, hospitals and urban air, relatively few studies have reported comprehensive community-level sequencing data for laboratory animal or animal testing facilities. Available publications on laboratory animal environments tend to focus on culture-based monitoring, indicator organisms or targeted pathogens relevant to animal health and biosecurity, rather than whole-community profiling (Lipman & Leary, 2015:1588–1589; Gilbert & Hartmann, 2024:742). Where molecular methods are used, they are often limited to surface sampling or specific matrices such as bedding or cage dust, rather than integrated air and surface metagenomic assessments (Gangneux *et al.*, 2020:1). Recent advances in air metagenomics using long-read sequencing have demonstrated the feasibility of species-level community profiling in low-biomass air samples from controlled and urban environments (Reska *et al.*, 2024:1) however, comparable studies within animal testing facilities remain limited. As a result, it is not yet well established whether animal facilities exhibit distinct microbial community signatures compared to other occupied indoor environments, or whether their profiles are primarily driven by the same factors of human occupancy, ventilation and cleaning practices.

3.5.2.3 Identification based on Nanopore sequencing

The Hazardous Biological Agents Regulations, 2022 of South Africa identifies four risk groups according to the probability of causing human disease, infecting the community and if adequate prophylaxis and treatments are available (Department of Employment and Labour, 2022:4). Risk group 2 agents may be hazardous to exposed persons and cause human disease but are unlikely to spread in the community and effective prophylaxis and treatments are generally available. Risk group 3 agents present a serious hazard to exposed persons and may cause severe human disease. They also present a risk of spreading to the community, however, effective prophylaxis and treatments are available. A total of 59 HBAs that fit into risk group 2 were identified in the facility, 53 of them bacterial and six of them fungal (Table 3.3). Some of these bacterial agents were also found to be toxin-producing, such as *Clostridium tetani,* known to cause tetanus, *Staphylococcus aureus,* known to cause pneumonia and *Vibrio cholerae,* known to cause cholera. Three agents were identified from risk group three: *Bacillus anthracis*, known to cause anthrax, *Shigella dysenteriae*, known to cause shigellosis (diarrhoea) and *E. coli,* known to cause gastrointestinal infections, urinary tract infections and food poisoning. *Bacillus anthracis* was found on the contact plates in the washing area, cloak room and inactive experimental room, in the microbial air samples in the experimental room and on surface swabs in the cloak room. DNA sequences that align with *Bacillus anthracis* were identified. However, given that this species is part of the *Bacillus cereus sensu lato* group, a complex of very closely related species whose 16S rRNA gene sequences share >99 % similarity and historically cannot be reliably differentiated by conventional 16S markers alone (Bavykin *et al*., 2004: 3712), the result cannot be considered definitive proof of the presence of *B. anthracis*. Due to the unreliability of

the method, further sample testing and analysis should be conducted to be able to confirm the identity of *B. anthracis*. *Shigella dysenteriae* was found in the microbial air samples in the cloak room, and *E. coli* was found in all samples except for settle plates in clean hallway 1 on the first day and the cyclone samples. The abundance of *E. coli* could be due to contamination, most likely from the dirty bedding, being spread throughout the facility. The spread could be, as explained above, the workers stepping in the dirty bedding or particles settle onto their person and spread as they move through the facility.

The Hazardous Biological Agents Regulations of South Africa (2022) specify the strains and types of certain agents, specifying verocytotoxigenic strains for *E. coli* and "type 1" *Shigella dysenteriae* classifications, both of these specifications producing Shiga toxin, which could cause serious health effects (Bennish & Khan, 2013:454; Messens *et al*., 2015:1652). However, the sequencing workflow did not provide specifics such as strains or types for these two agents. They were thus included on a precautionary basis (Department of Employment and Labour, 2022:29). The presence of these HBAs, even slight, warrants concern and increases the need for better control and mitigative measures.

The assessment of risk can be difficult concerning exposure to HBAs, because the type of agent indicates the risk to human health and also the risk of spreading to the community, rather than the dose. Consequently, being exposed to the smallest amount of HBAs could cause human disease that could spread rapidly throughout the community (Whitby *et al*., 2022:104–105). The presence of various risk group 2 agents increases the likelihood of a person being exposed to a health hazard. Additionally, when risk group 3 agents are present, which can cause serious human disease, the health risks associated with exposure are heightened. Considering the potential presence of *Bacillus anthracis* in this study, within the field of Occupational Hygiene requires that risk estimation, assessment and mitigation be based on the "worst-case scenario." This means that the evaluation of risk and the resulting recommendations must proceed under the assumption that the pathogen may be present within the facility, until proven otherwise (Spear, 2005:39).

The *Homo sapiens* DNA identified throughout the facility could be explained by either possible contamination from the researchers who executed the sampling and analysis, an unlikely scenario as all measures were followed to mitigate any possible contamination. The other more likely option is the accumulation of DNA from hair and skin cells that are shed by the workers in the facility. This is further supported by the fact that the settle plate and surface swab samples showed the highest amounts of *Homo sapiens* DNA, in contrast to the low concentrations detected by the microbial air sampler. (Figure 3.22). Meadow *et al*. (2015:1) assessed that humans emit upwards of 10^6 biological particles per hour. This happens in three different ways: first is direct contact on surfaces, second emissions from breath, clothes, hair and skin, and lastly the resuspension of dust particulates containing the previously mentioned human hair, skin cells and other microbes. The use of PPE should reduce the amount of these biological particles being shed, specifically when wearing surgical gloves and masks,

hair bonnets and lab coats. However, human nature should still be taken into account, meaning that the workers might not be wearing the PPE correctly or even at all. Even though surgical masks are effective at capturing droplets from the nose and mouth, they do not cover the entire face or neck areas and do not capture all skin cells and droplet particles, as many can still escape via the gaps on the sides of the masks (Coffey *et al*., 2020). When taking these facts into account and the fact that the facility is only cleaned once a month according to their standard operating procedures, it provides ample time for the accumulation of human biological particles.

3.5.3 Environmental parameters

The facility's environmental parameters, such as temperature and RH, are carefully regulated according to the guidelines for animal testing facilities, which can vary depending on the requirements of the research or specific animals. The University of California, Los Angeles (UCLA) (2025) provides guidelines for specific animals housed in the facility. In the case of this specifically sampled facility, the temperature should range between 20–26°C and RH 30–70%. The specific facility sampled in this study aims for the RH to remain under 70% and temperatures approximately at 22°C (Bester & Minnaar, 2023). The Department of Occupational Safety and Health of Malaysia (2019) outlines guidelines for indoor air quality (IAQ) parameters, such as temperature (23–26°C), RH (40–70%), air velocity (0.15–0.50 m/s), CO concentration (< 10 ppm), CO_2 concentration (< 1000 ppm), respirable particulates (< 0.15 mg/m^3), bacterial count (< 500 CFU/m^3) and fungal count (< 1000 CFU/m^3).

The temperatures throughout the facility remained relatively stable, with the cloak room and washing area providing the highest and lowest readings of the facility, respectively. The RH varied throughout the facility, peaking in the cloak room and washing area. The high temperature and RH levels in the cloak room could be because this room is not connected to any type of mechanical ventilation system. The washing area, although connected to the ventilation system, does not form part of the clean facility that is carefully controlled. Kalyoncu (2010:553–557) assessed the impact of environmental factors on fungal spore growth. It was found that fungal growth preferred a range of 9.5–32.4°C for optimal growth, and Lyubetsky *et al.* (2020:1) found that bacterial growth preferred a range of 7–85°C (all depending on the type of microbe). Even though previous publications found that temperature could influence microbial growth, no significant correlations were found between the microbial growth and temperature in the facility during the current study. Kalyoncu (2010:556) could correlate the RH to the microbial growth, as the RH increased, so did the microbial concentrations. Within this study, no statistically significant correlations were found between the RH and the microbial growth in the rooms, differing from the results obtained from Kalyoncu (2010:553–557). The facility requires that all rooms remain in a positive pressure state (Bester & Minnaar, 2023). Air velocity in all the rooms were low with results below 0.1 m/s, which could indicate poor ventilation within the rooms, leading to larger particles settling quicker and smaller particles remaining airborne longer (as seen from the dust

particulate matter results) (Worrell, 2024). Kalyoncu (2010:556) found no correlation between air velocity and microbial concentration, which was corroborated with the results obtained from this study. The levels of CO and CO_2 found in the facility were overall within levels considered to be safe (CO < 10 ppm and CO_2 < 1000 ppm) (Department of Occupational Safety and Health of Malaysia, 2010). However, higher CO_2 levels in the cloak room, breeding room and experimental room (> 500 ppm) could be due to human and animal occupancy of the area. The washing room yielded overall the highest dust particulate matter concentrations over all three days, specifically in the size fractions of ≤ 0.375 µm. No statistically significant correlations were found between the dust concentrations and air microbial growth concentrations. It is important to note that poor ventilation could lead to an increased risk of exposure to dust particulates carrying HBAs since the larger particles settle, leading to surface contamination and the smaller particles remain airborne, increasing the risk of inhaling particulates that can penetrate deep into the lungs (Brown, 2013:1).

3.6 Conclusion

Based on both culture-based and metagenomic results, a wide variety of HBAs were identified throughout the animal testing facility, including agents categorised as risk group 2 and 3 under the Hazardous Biological Agents Regulations, 2022 of South Africa (Department of Employment and Labour, 2022:24–30). The microbial air sampler yielded the highest microbial concentration and alpha diversity indices when compared to that of surface swabbing samples, which are considered the standard procedure when sampling for HBAs. This highlights the importance of using various sampling methods when assessing HBAs to capture all microorganisms present in the air and on surfaces. The cyclone sampler could capture not only culture-independent microbes otherwise missed with the other sampling methods, but also size-specific microbes which could help to determine and assess the exposure risk to HBAs when used correctly.

When assessing the risk of exposure to HBAs, it is important to note the route of exposure, the type of agent and the control measures in place to prevent exposure. The most abundant size dust particles present in the facility were those of the smallest sizes sampled (0.300 µm and 0.375 µm), meaning that if any of these dust particles were inhaled while carrying any of the HBAs identified, they would have direct access to the gas exchange region of the lungs to cause infection. As discussed, although the abundance of microbial growth concentration might be low, the variety of risk group 2 HBAs found in the facility increases the likelihood of exposure, and the inclusion of risk group 3 and toxin-producing agents increases the potential severity of exposure. This would lead to an assumption of a high-risk exposure scenario, due to the variety of risk group agents found: 53 bacterial and six fungal risk group 2 HBAs, three of which are toxin-producing agents and three risk group 3 HBAs, where the three risk group 3 agents pose serious health risks to exposed workers. When taking the control measures in place into consideration, PPE such as the surgical mask may not mitigate the inhalation of HBAs due

to gaps on the sides of the mask, which may let in unwanted particles, but it does decrease the probability of inhalation of larger particles (Coffey *et al*., 2020). Surgical gloves and lab coats protect the skin of the hands and arms against any surface contamination from the larger settled particles. It does, however, not protect the exposed areas such as the face, neck and wrists.

When assessing the occupational exposure risks of HBAs in an animal testing facility, a few factors should be taken into account. As mentioned above, the major route of exposure within this facility is that of inhalation of bioaerosols, and the variety of HBAs provides opportunities for severe human disease. However, the likelihood of harm being caused can depend on the workers' immune system and bodily defence mechanisms, for example, persons with allergies or who are easily sensitised are more susceptible to HBAs than those with strong immune systems. Even though the abundance of HBAs does not affect the severity of exposure, it does determine the likelihood of exposure. The more HBAs present in an area, the more likely workers will be exposed. Within this facility, the level of microbial growth is relatively low with a few outliers, indicating a lower likelihood of exposure. The ventilation system within the facility is adequate in providing uncontaminated air, however, inadequate in removing contaminated air, and even with the addition of the PPE, such as the surgical masks, and the exposed skin areas, could increase the risk of exposure to the HBAs found in the facility. When taking all of these factors into account, an assessment can be made that the HBAs found in this animal testing facility pose a moderate risk to the workers' health.

This study not only found the presence of HBAs in a facility with strict protocols to limit the presence of unwanted microbes, but also identified HBAs that are listed as risk group 2 and 3 according to the Hazardous Biological Agents Regulations, 2022 of South Africa. These are agents which could cause serious health effects if not correctly controlled or mitigated.

Furthermore, this study highlighted the importance of including multiple sampling strategies when sampling for HBAs. When only conducting surface sampling, it excludes a major abundance of microbes that could be present in the room and underrepresents the true concentration of microbes in an area. By including an air sampler, such as the MAS, the airborne HBAs posing a risk to be inhaled are also assessed. The MAS proved to be effective at sampling occupational settings and providing the highest diversity of microbes with further genomic analysis, providing a better picture of the true exposure to HBAs in the workplace.

3.7 References

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CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

In this chapter, an overall summarised conclusion and key findings of this study are provided. The aim and objectives of this study were addressed, and the research question was answered.

4.1 Conclusion

This study aimed to determine the presence of hazardous biological agents within an animal testing facility, as well as to identify these agents by completing four objectives.

The first objective stated, '*To perform passive surface sampling and active air sampling within selected areas of the animal testing facility, to determine the presence of potentially hazardous biological agents*.' This objective was achieved by conducting active and passive microbial sampling in various rooms of the facility, providing air and surface microbial growth concentrations in each room. The cloak room proved to be the area with the highest microbial growth overall, followed by the breeding room, experimental room, washing area and hallways. The microbial air sampler yielded the best results compared to the other sampling methods. However, important data were still obtained from the other sampling methods, indicating the importance of including various sampling methods when collecting for HBAs. The results confirmed the presence of various biological agents in the animal testing facility, some of which are potentially hazardous. The abundance of some of these biological agents in some areas of the facility is important to note. Specifically for identifying areas that are contamination sources, where the continuation originated from as well as how to mitigate or control the amount of biological agents found. This is important, specifically in a facility that strives to minimise the amount of outside biological agents and pathogens from entering the facility.

The second objective stated, '*To identify the hazardous biological agents collected in objective 1 by use of genomic analysis and categorise the HBAs into their respective risk groups according to the South African Regulations for Hazardous Biological Agents, 2022*' This objective was achieved by identifying the HBAs down to the genus and species level by conducting genomic analysis of the samples obtained from the first objective. A total of 59 risk group 2 and three risk group 3 HBAs were identified across the animal testing facility, where five of these agents are toxin-producing. The abundance of risk group 2 HBAs within the facility poses a high risk that workers may be exposed to one of these agents. When considering the presence of risk group 3 HBAs, a few toxin-producing agents, and the inadequate protection provided by surgical masks as PPE, a high health risk is posed to the workers. Furthermore, the use of genomic analysis offers a significant advantage in the field of occupational hygiene, as it enables the accurate identification of HBAs, rather than relying solely on growth morphology and microbial quantification. Techniques such as nanopore sequencing provide

detailed taxonomic information, allowing for a more informed assessment of the true health risks associated with exposure to HBAs.

The third and fourth objectives stated, '*To measure physical parameters such as airflow velocity, carbon monoxide and carbon dioxide concentration, temperature and relative humidity and furthermore to correlate the values with the concentrations of HBAs found, since these factors may influence the presence of hazardous biological agents in the animal testing facility*' and '*to determine the presence of dust particulate matter by real-time sampling and to correlate its presence in relation with the concentration of HBAs found, since particulate matter acts as carriers of HBAs*'. These objectives were achieved when correlation tests were done between the environmental (physical and chemical) parameters and the concentrations of microbial growth in the different rooms of the animal testing facility. No statistically significant correlations were found between the environmental parameters and microbial growth concentrations. This could be due to the facility carefully controlling the environmental factors. These factors remained mostly in close range of one another and within the standards outlined by UCLA (2025) for these types of facilities. The concentration of dust particulate matter was insignificant. However, it was observed that the concentrations of dust were the highest within the smallest size fractions (< 1 µm) of dust particulates. Even though the results were not statistically significant, literature on the topic highlights that it is still important to include these factors when assessing microbial contamination, as these factors could influence the presence and growth of HBAs.

Lastly, the research question of this study was: *'To what extent are the HBAs found in the animal testing facility hazardous to the workers' health, as categorised into the risk groups according to the South African Hazardous Biological Agents Regulations, 2022?'*

To be able to answer this question, a few factors should be considered. First, the biological agents found in the animal testing facility are hazardous, according to the variety of risk group 2 agents and the presence of risk group 3 agents that were identified as outlined by the South African Hazardous Biological Agents Regulations, 2022 (Department of Employment and Labour, 2022:23–30). Furthermore, the microbial growth concentrations found were low when compared to IAQ standards (< 500 CFU/m^3 bacterial count and < 1000 CFU/m^3 fungal count) outlined by the Department of Occupational Safety and Health of Malaysia (2019). However, when looking at the small size of dust particulate matter present within the facility, including the lack of airflow within the rooms, the risk of exposure increases as the small particles stay airborne for long periods due to inadequate ventilation (Brown, 2005:831). These small particles, including microorganisms, have the potential to move through the openings on the sides of the face when wearing a surgical mask and penetrate deep into the lungs where air exchange takes place, if inhaled by the workers. Considering all these aspects, it can be concluded that the risk of exposure to the HBAs present in this animal testing facility is

moderate. This means that the HBAs could pose a risk to the workers' health if not correctly controlled or mitigated. As a result, although the risk level is moderate, the priority for the facility should be high and should be addressed as soon as possible, particularly to prevent this risk from escalating and to ensure long-term protection of the workers' health.

The inclusion of new technologies such as nanopore sequencing to conduct identification of microbes in the field of occupational hygiene is still a novel approach. This study highlighted how genomic analysis can improve risk estimation by providing the identity of HBAs. The identified HBAs can be classified into their respective risk groups according to the South African Hazardous Biological Agents Regulations (2022), providing better insight into the true extent of the health hazards associated with specific HBAs. However, the limitations highlighted in this study must also be taken into account, and appropriate mitigating measures should be implemented to eliminate or work around the constraints of this method.

4.2 Recommendations

In this section, recommendations are provided for the animal testing facility to decrease the workers' risk of exposure to HBAs, as well as recommendations to be included in future studies.

4.2.1 Control measures

No eliminations or substitutions can be made to decrease the presence of HBAs in the animal testing facility. This is due to the omnipresence of HBAs from all outdoor, indoor, living and non-living sources, making elimination not possible, unless implementing BSL-4 laboratory measures, which is not reasonably practicable for a BSL-2 facility. Substitution is also not possible due to the HBAs being the subject of research in many of these facilities.

4.2.1.1 Engineering controls

The most effective control measure that can be recommended to decrease the risk of exposure to HBAs is upgrading the ventilation system so that it effectively extracts the air from the areas. Then the HBAs can be successfully removed via the extraction ventilation. Regular testing and maintenance should also be conducted to ensure that all the equipment is working effectively and not possibly increasing the risk of exposure (Department of Employment and Labour, 2022:18). Install an ultraviolet germicidal irradiation (UVGI) system in the cloak room, specifically to decontaminate the lab coats regularly used throughout the day (CDC, 2024a). To decrease the contamination of the clean floor from the dirty floor, a disinfecting shoe bath can be utilised in conjunction with the disposal of old shoe covers and donning new shoe covers when entering the clean facility from the dirty, using the same procedure when entering the facility (SupplyWise, 2025).

4.2.1.2 Administrative

Update the standard operating procedures (SOPs) if any changes have occurred in the facility or problems have been identified. Changes could include more regular cleaning of the facility, such as once a week instead of once a month. Introduce an antimicrobial soap and sanitiser such as a 70% ethanol solution to the cleaning procedure. Conduct regular training regarding topics such as the updated SOPs, the donning, doffing and wearing of PPE, as well as the type of hazards present in the facility. Provide specific training to the cleaning staff, who are usually poorly educated with regards to these hazards, about the facility and regarding the type of hazards found in the facility and health implications connected to their exposure. Educate them as to why it is important to follow the proper cleaning procedures and the possible negative effects when not executed correctly. Keep a record of work-related or medically significant incidents at the facility, for investigatory and traceability purposes. Lastly, include sanitation stations in each area, so that they are readily available when needed.

4.2.1.3 Personal protective equipment

PPE is always used as a last resort, when following the hierarchy of control, due to it only being a barrier between the worker and the hazard and not actually mitigating the hazard (CDC, 2024b). Furthermore, the effectiveness of PPE is dependent on the worker wearing it consistently and correctly (George *et al*., 2023:1). It is therefore a protective measure that is only effective with cooperation from the worker. Replace the surgical masks with FFP2 respirators, specifically for the workers who clean the animal cages, those who work with the dirty bedding and those who work in the breeding room, as these respirators would provide adequate protection against any airborne contaminants. In addition, respirator fit testing needs to be conducted to ensure that the worker is wearing the correct respirator for their face shape and is also wearing it correctly. The respirator fit test would have to be repeated regularly, for example, when a new worker joins the facility, respirator brands change, problems have been noticed by staff and not exceeding 24 months (Department of Employment and Labour, 2022:19).

4.2.2 Future studies

The following are recommendations to take into account when conducting similar studies in the future:

- Conduct personal sampling for HBAs to assess individual exposure risks to workers during different activities.
- Sample twice a day, once in the morning and once in the evening, to obtain enough data to conduct accurate statistical analyses between the environmental parameters and the microbial growth.
- Collect samples of the animal bedding to be sequenced, to be able to identify the HBAs that become airborne when the dirty bedding is being handled.
- Leave the cyclone sampler to run for the entire working day to collect representative samples of the workers' shift.
- Leave the settle plates out for four hours to collect representative samples.
- Collect additional cyclone samples to collect RNA, specifically to test for the presence of viruses.
- Assess how workers who have allergies or are easily sensitised are influenced by their working environment, specifically about HBAs.
- Collect additional surface samples in the larger rooms, a minimum of five samples to be able to accurately identify contaminated surfaces.
- Conduct a more in-depth assessment of the ventilation system, such as measuring face velocity of the vents in the areas, as well as determining the air exchange rate.

4.3 References

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CHAPTER 5 APPENDICES

5.1 Supplementary material

5.1.1 Detailed description of materials and methods

5.1.1.1 Facility description

A walk-through survey was conducted to get familiarised with the facility's layout and control measures, as well as to identify possible sources of exposure and sampling areas. The facility's layout follows the concept of separate clean and dirty areas where workers are restricted to move to a clean area after being in a dirty area (Figure 3.2). The facility utilises only mechanical ventilation through a centralised heating, ventilation, and air conditioning (HVAC) system that controls the temperature, relative humidity and the positive pressure within the rooms. Basic PPE, such as laboratory coats and disposable gloves, is worn with additional PPE needed to protect the integrity of the research. The facility is cleaned once a month, and the general cleanliness of the rooms are maintained by the researchers, for example, cleaning the workbenches after examining the animal test subjects. After the survey was conducted, the following rooms and areas were identified for sampling:

Cloak room: This room has a surface area of 1.5 m^2 and contains all the appropriate PPE required to be donned before entering the facility. The cloak room is separated into a clean and dirty area via a line on the floor. This line is the indicator used when entering and exiting the clean area of the facility. Lab coats are the only non-disposable items that are provided to visitors and other staff members. The facility has a system in place where staff lab coats are separated from the visitors' lab coats and coats are placed into clean or dirty bins. The dirty bin is in the hallway outside the cloak room. When a person exits the facility, all PPE are doffed and discarded into a biohazardous waste bin, and the lab coats are placed into the dirty bin. The dirty lab coats are washed and placed in the clean bin to be used again.

Experimental room: This room, with a surface area of 10.6 m^2, is where active experiments are conducted. This room usually consists of animal cages, stainless steel work benches, storage cupboards housing experimental equipment and a washbasin. Unfortunately, due to the sensitivity of the animal test subjects and the risk of introducing unwanted outside pathogens, entrance into active experimental rooms was not permitted. An inactive experimental room was sampled as a baseline exposure assessment.

Breeding room: This room has a surface area of 66.9 m^2 and is occupied by approximately 280 animal cages housing (cage dimensions approximately 29cm L x 19cm W x 13cm D), on average, five animals each. This area is specifically used to house and breed the animals used in the experiments.

Hallways: The facility is divided into three areas, where clean hallway 1 gives access to the experimental rooms and breeding room and connects to clean hallway 2. Clean hallway 2 leads to the conventional washing area and the elevator that provides access to the washing area in the basement and leads to the start of the dirty area. One needs to move through the conventional washing area and clean cage storage to access the dirty hallway. Due to the layout of the facility, one would need to enter the clean hallway from the dirty hallway again to exit the facility. A rule was implemented where if a person needed to exit the building, they are permitted to move into clean hallway 1 from the dirty hallway, provided that they do not enter any experimental rooms, to reduce the risk of contamination (Figure 3.2).

Washing area: This room has a surface area of 63.6 m^2 and is situated in the basement of the facility. The basement is not included in Figure 3.2 because it does not form part of the flow pattern of the main facility. The area contains an autoclave, two cage washers connected to a steam producer, multiple deep washbasins and a washing machine and dryer for the lab coats. Laboratory coats and surgical gloves are worn in this area of the facility. The dirty cages and water bottles are transported from the main facility above via an elevator. The animal cages and water bottles are cleaned and sanitised in this part of the facility before they can be reused. Although standard practice indicates that the dirty animal bedding is removed and placed in the conventional washing area in the facility above and later disposed of in a designated area outside of the facility, a biohazardous waste bin with dirty bedding was observed in the washing area. This indicates a possible source of HBA exposure, as dirty animal bedding should not be present in this part of the facility.

5.1.1.2 Agar plate preparation

For this study, four different types of agar for sampling HBAs in the animal testing facility were used. Standard count agar, which will be referred to as nutrient agar (NA), for growth of non-specific microbes (Condalab, 2022); potato dextrose agar (PDA) for specific growth of yeasts and moulds (Thermo Fisher Scientific, 2007); GranuCult® ENDO agar for specific growth of *E.coli* and coliforms (Millipore, 2019) and blood agar for specific testing of the haemolytic activity of the microbes. Laboratory coats and surgical gloves were worn during the entirety of the process, and 70% ethanol was used to sanitise the gloves, equipment and working surfaces during the preparation process. The dry agar powder was weighed according to the measurements provided by the manufacturers, 25 g of NA and 39 g of PDA and ENDO agar, using an electronic analytical balance. The powders were

each individually mixed in an autoclavable glass bottle with one litre of distilled water and shaken until all the agar powder was dissolved. The agar mixtures were then autoclaved at 121°C for 15 minutes (Condalab, 2022; Thermo Fisher Scientific, 2007; Millipore, 2019). The bottles with agar mixtures were moved to a preheated water bath that was set to 50°C to ensure that the agar mixture remained in a liquid state. A laminar flow cabinet was used to provide a sterile area for the pouring procedure and the surface was sterilised with a 70% ethanol solution before use. The packaging containing empty 90 mm and 50 mm agar plates was sanitised with 70% ethanol and placed in the cabinet, where the packaging was cut open and placed aside in the cabinet for later use. The bottles of agar were removed one at a time from the water bath, dried and placed in the cabinet. The agar mixtures were poured into the 90 mm plates, ensuring that the bottom of the plates was covered and the agar reached the bottom of an indication arrow on the side of the plate. The 55 mm contact plates were poured in such a way that the agar formed a convex shape at the rim of the plate (Petersen *et al.*, 2016:2–3). The plates were then left to cool and the agar to solidify, after which the plates were labelled with the type of agar. The plates were then placed and closed in the original packaging and stored in a fridge until use.

5.1.1.3 Surface swabbing

This sampling method employed a 10 x 10 cm template to maintain uniformity across all samples . The sampled surfaces included the workbenches, cupboards housing PPE, floors and the surfaces in and around washbasin openings. The air supply ventilation openings (AC vents) were also swabbed; however, due to the shape of the openings, a template could not be utilised, and an estimated area was swabbed close to the surface area of the template. The templates were cleaned: firstly, being washed with warm water and antibacterial soap, and secondly being dried and sprayed with 70% ethanol and placed in a clean plastic bag that was also treated with the same process. Disposable gloves were donned before sampling and worn during the duration of sampling. A solution of 70% ethanol was used to sanitise the gloves before and after each sample collection. After a sampling area was identified and noted on the field sheets, a template was placed on the sampling surface. Then a sterile cotton swab was removed from its container, breaking the sterile seal. A specific swabbing method was followed to ensure maximum surface area coverage, following a pattern of moving up and down, left to right, then diagonally, and finally diagonally in the opposite direction (Figure S 1). This was done while also ensuring that the swab was rolled and held at a 45° angle to provide maximum coverage of the swab. The swab was then placed back into its tube and labelled with the sampling location and date. The dirty templates were kept separate from the clean ones for another round of cleaning. The swabs were transported back to the laboratory, where the swab contents were transferred to the appropriate agar plates by swabbing in a zig-zag pattern, ensuring the swab was rolled and held at a 45° angle. The sampled area and date of the swab sample

were written on the agar plate. The agar plates were then inverted and incubated for 48 hours at a constant temperature of 37°C (Hanlon & Hodges, 2013:18).

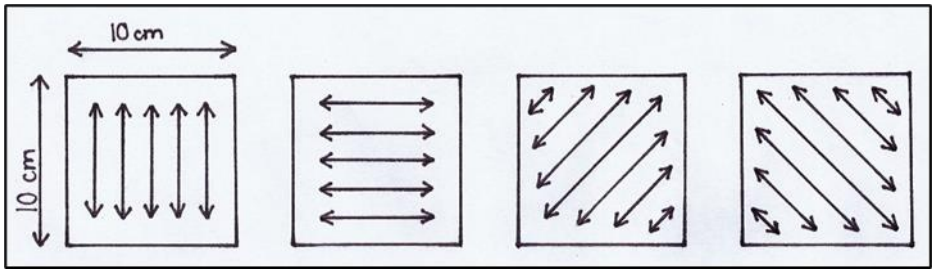


Figure S 1: Surface swabbing pattern adapted from Cole-Parmer (2018).

5.1.1.4 Contact plates

Contact plates were used to gather samples from unique and uneven surfaces due to the convex shape of the agar. These sampled surfaces included areas in and around washbasins, extraction ventilation openings (EX vents), animal cages, door handles, as well as lab coats. Disposable gloves were donned before sampling and worn during the duration of sampling, while a solution of 70% ethanol was used to sterilise the gloves before and after each sample collection. The plate was opened, and the agar plate was pressed against the sampling surface, ensuring full coverage of the plate by rolling the plate over the surface. The plate was then closed and labelled with the sampling location and date. The plates were then transported back to the laboratory, inverted and incubated for 48 hours at a constant temperature of 37°C (Hanlon & Hodges, 2013:18).

5.1.1.5 Settle plates

Standard 90 mm agar plates that are used to collect settling airborne microbes directly onto the plate are referred to as settle plates (NIOSH, 2017:23–24). These agar plates were placed in the hallways of the facility. The plates were opened and placed collection-side up on the floors of the hallways. The settle plates were left open for collection for 1–1.5 hours (in contrast to the standard procedure of 4 hours (Eaton, 2022:2)), due to the limited time the researcher had access to the facility for. The plates were then closed and labelled with the sampling location and date. The plates were then transported back to the laboratory, inverted and incubated for 48 hours at a constant temperature of 37°C (Hanlon & Hodges, 2013:18).

5.1.1.6 Microbial air sampler

The MAS-100 NT® is a microbial air sampler (MAS) that captures bioaerosols directly onto an agar plate. The sampler was pre-calibrated by the manufacturer to sample for 10 minutes at a flow rate of 100 L/min (Millipore, 2020:2). The sampled areas included the cloak room, experimental room, breeding room and washing area, where the sampler was placed on the working benches in the rooms facing in the direction of the working area to capture the air that the workers could inhale. Disposable gloves were donned and sanitised with a solution of 70% ethanol before sampling and between each sample. The lid of the MAS was removed, and the sampling base as well as the perforated lid of the sampler were sprayed and wiped down with a 70% ethanol solution. The agar plate was placed onto the base of the sampler, and the perforated lid was used to close the sampler. The arm of the sampler was placed in an upright position and left to run for 10 minutes per agar plate. After the run time was completed, the agar plate was removed from the sampler, closed and labelled with the sampling location and date. The sampling base and perforated lid of the MAS were sprayed and wiped down with a 70% ethanol solution before and after each sample. The plates were then transported back to the laboratory, inverted and incubated for 48 hours at a constant temperature of 37°C (Hanlon & Hodges, 2013:18).

5.1.1.7 Bioaerosol cyclone

The TE-BC251 bioaerosol cyclone is a culture independent sampling method that can collect size specific bioaerosols. The sampler draws in air through a 15 ml tube to catch particles larger than 4 µm. The air then moves to a 1.5 ml tube that captures particles from 1 to 4 µm and the final section contains a filter that captures particles smaller than 1 µm. This method is different to that of the MAS due to the ability to sample size specific particulates and can be sequenced without needing to culture the microorganisms first (NIOSH, 2020:1–2). The cyclone went through a sterilisation process where it was washed in an ultrasonic bath with soap for 1 hour, sprayed with a solution of 70% ethanol and wrapped in aluminium foil to be autoclaved at 121°C for 15 minutes and left in an oven to dry. Once the cyclone had fully dried, it was transferred to a laminar flow cabinet where the O-rings were placed onto the cyclones, where they were attached to the cassettes and then closed again with the aluminium foil. The 3-piece filter cassettes were not suitable for autoclaving and were sterilised with a 70% ethanol solution and assembled with a 0.8 µm pore size mixed cellulose esters (MCE) membrane filter on top of a support pad. Lastly, the GilAir plus personal sampling pumps were calibrated to a flow rate of 3.5 L/min (NIOSH, 2018:6–15). All sample materials and accessories were transported to the sampling locations, and the sampling train was assembled on-site. The sampled areas included the breeding room and washing area based on the assumption of high microbial presence there. The cyclone was attached to a tripod standing approximately 1 m tall and left to run for approximately 45 minutes per area. When the sampling time was completed, the filter cassette

and sampling tubes were removed from the cyclone, sealed and marked with the sampling location and date. The tubes and filter cassette were transported back to the laboratory, where the filters were removed and placed in a separate tube and stored in a freezer until the samples could be sequenced.

5.1.1.8 Aerosolised particulate matter

The Nanozen DustCount 9000 is a handheld direct reading instrument that detects the dust particulate matter concentrations in real time, as well as simultaneously establishing particle size distribution over the selected size fraction (Nanozen, 2019:12–15). The Nanozen DustCount was cleaned before it was used for sampling. The inlet nozzle that houses the impactor was removed. The impactor was cleaned by lightly dabbing it with an alcohol swab and left to dry. While the impactor was drying, the device was turned on and cleaned by applying compressed air to the inlet. After the impactor had dried, a small drop of oil was added to the impactor and left to soak for a few seconds. Once the oil had fully saturated the impactor, the surplus was removed using the tip of a paper towel. All was reassembled and sampling could commence. The instrument was automatically set to a flow rate of 1 L/min. The areas sampled included the cloak room, experimental room, breeding room and the washing area placing the instrument on the workbenches, where it was most likely to collect the air that the workers could inhale. The pump was switched on and left to run for approximately 45 minutes per area. The pump was transported back to the laboratory, and the data was downloaded from the instrument in the form of a Microsoft Excel spreadsheet. The Nanozen DustCount 9000 provides an average concentration per sampled area in mg/m^3 as well as the particle count concentration of various size fractions (0.3–2.793 µm) in 1000 counts/m^3.

5.1.1.9 Physical parameters

The TSI IAQ-Calc™ Indoor Air Quality Meter 7545 (TSI Incorporated, United States of America) is a handheld device that measures the physical parameters in the environment. These parameters include carbon monoxide (CO) concentration, carbon dioxide (CO_2) concentration, temperature and relative humidity (RH) (TSI, 2022:3–7). The sampled areas included the cloak room, experimental room, breeding room, washing area, both clean hallways 1 and 2, as well as the dirty hallway. Measurements were taken by switching on the device, leaving it for a few seconds to stabilise, then pressing the enter button and waiting for five seconds. The device then displayed the values for all the above-mentioned variables. An average of three to four measurements was taken within the areas with each measurement noted down on the field sheets. Due to the large dimension of the breeding room, it was divided into 1 x 1 m squares, with three readings taken in each square.

5.1.1.10 Air velocity

The TSI Airflow™ TA465-P Air Velocity Meter (TSI Incorporated, United States of America) is a handheld device that is used to measure the airflow and face velocity of ventilation systems (TSI, 2024:5–16). A zero check was conducted to ensure the accuracy of the measurements before sampling. The sampled areas included the cloak room, experimental room, breeding room, washing area, both clean hallways 1 and 2, as well as the dirty hallway. Measurements were taken by switching on the device, leaving it for a few seconds to stabilise, then pressing the enter button and waiting for five seconds. The meter then displayed the airflow of the room in m/s. An average of three to four measurements was taken within the room; however, due to the large surface area of the breeding room, the area was divided into 1 x 1 m squares where an average of three readings per square was taken. Each measurement was noted down on the field sheets.

5.1.1.11 DNA extraction

Before the sequencing protocol could be followed, the deoxyribonucleic acid (DNA) had to be extracted from the growth on the incubated agar plates from both the passive and active biological sampling methods, as well as the sampled tubes from the cyclone sampler using a NucleoSpin® Soil kit (Macherey-Nagel, 2024) and the manufacturer's instructions. Only a few of the samples were subjected to extraction and sequencing. These samples were chosen based on the abundance of growth and morphological characteristics. The samples were grouped according to sampling type, room and day. This entire process was conducted within a laminar flow cabinet, and the cabinet was sanitised with a 70% ethanol solution before and after use to ensure a clean and sterile environment inside the cabinet. These components included a pipette stand, pipettes, containers holding pipette tips, test tube racks, a black marker, waste containers, as well as various bags containing sterile test tubes and bottles containing liquids from the kit. Laboratory spatulas were washed with antibacterial soap, rinsed with distilled water and sanitised with a 70% ethanol solution before they were used to scrape off the growth from the agar plates. The scrapings were placed into a tube containing ceramic beads, a lysis buffer (SL1) and enhancer (SX) from the kit to break down the samples. The contaminants in the samples were precipitated by adding another lysis buffer (SL3) from the kit, followed by an incubation period at 0–4°C and centrifuging at 4 000 g (gravitational force) to provide a clear supernatant. In the case where the supernatant wasn't fully clear, it was pipetted to a clean tube where it was centrifuged again. The clear supernatant was pipetted into a column (red) containing a filter that removes inhibitors (Figure S 2). The binding condition was adjusted after the supernatant was filtered through the column using centrifugal force, by adding a binding buffer (SB). The sample was then transferred to another column (green) containing a silicate membrane filter and was centrifuged (4000 g); all flow-through from this column was discarded as the DNA had bonded to the filter in the column. The filter underwent a five-step washing and drying cycle. An elution buffer

(SE) from the kit was used to flow through the filter and bind to the DNA, leaving a sample of approximately 50 µl of DNA.

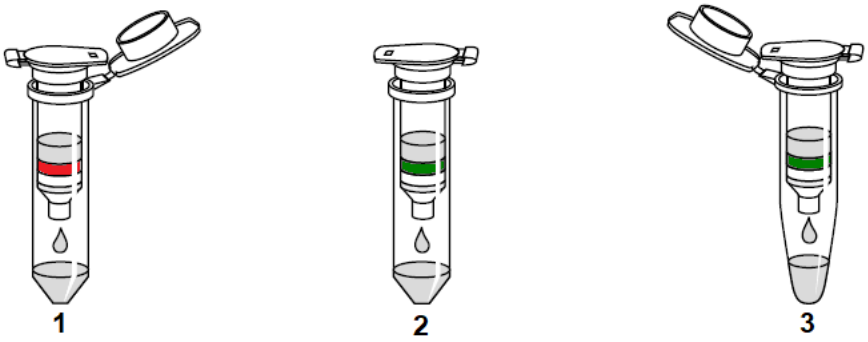


Figure S 2: The columns used during the DNA extraction workflow are tubes containing filters that fit in a standard 2 ml collection tube and make use of centrifugal force to allow the liquid sample to flow through the filter. Column one with a red ring is the column that removes inhibitors. Columns two and three, with a green ring each, are the same column but are used during different steps of the procedure. Column two illustrates the washing cycle and column three illustrates the flow through of the elution buffer that bonded with the DNA (Macherey-Nagel, 2024).

The NanoDrop Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Incorporated, United States of America) (Thermo Fisher Scientific, 2023:25–27) was used to test the quality (A260/A280 and A260/A230 values) and determine the concentration of the DNA in ng/µl. The machine was switched on and the dsDNA option was chosen, whereafter the instrument had to be blanked. The blanking was done by picking up the arm and cleaning the pedestal and arm using a Kimwipe™, a light-duty wipe used for delicate tasks (Kimberly-Clark Professionals, 2025). Then, 2 µl of the elution buffer was placed on the pedestal using a pipette and the arm was closed. After the instrument blanked successfully, the arm was lifted, and the elution buffer was wiped off using a Kimwipe™. The DNA samples were first centrifuged (4 000 g) for a few seconds, and then a 2 µl sample was pipetted onto the pedestal and the arm was closed, automatically starting the analysis of the sample. The instrument then provided the quality and concentration of the DNA, which was written down on the laboratory notes. The sample was wiped off with a Kimwipe™ and the next sample was loaded. This process was repeated for all 46 DNA samples. After testing was completed, the “end experiment” button was selected, and the arm and pedestal of the instrument were cleaned with a Kimwipe™ and switched off. The extracted DNA was then stored in a freezer until it could be sequenced.

5.1.1.12 Nanopore sequencing

The rapid sequencing DNA V14 barcoding kit (SQK-RBK114-24) was used for the specific workflow in this study (ONT, 2025). The workflow required a 10 µl sample containing 200 ng of DNA. The following formula was used to calculate the total volume of DNA needed:

$$Volume\ of\ DNA\ (\mu l) = \frac{200\ ng}{Concentration\ of\ DNA\ in\ ng/\mu l}$$

Secondly, the flow cell (Figure S 3) was checked to determine if the cell has at least 800 or more pores available, and lastly, all elements from the barcoding kit and DNA samples were left to thaw before use, because everything was stored at -20°C. In a 0.2 ml polymerase chain reaction (PCR) tube, the DNA was prepared, and nuclease-free water was added to adjust the total volume of the sample to 10 µl. In cases where the calculated volume of DNA exceeded 10 µl due to low DNA concentrations, the full 10 µl of undiluted DNA was used. The kit consists of 24 barcodes; one barcode was attached to one sample of DNA, and the barcode number and DNA sample number were noted. After the barcodes had been added to the samples, the tubes were incubated at 30°C for two minutes and then at 80°C for another two minutes. After the tubes had cooled down, all the barcoded samples were then pooled together in a clean 2 ml tube. An AMPure XP Beads mixture was added to the barcoded DNA, where the beads in the mixture bonded with the DNA. The beads are magnetic, and a magnetic rack was used to pool all the beads to one side of the tube so that the beads could be washed with an 80% ethanol mixture without disturbing or accidentally removing some of the beads. The beads were left to dry, but not dry to the point of cracking, after which an elution buffer (EB) from the kit was added to resuspend the beads. The beads were once again moved aside using the magnet, and the barcoded DNA that was now mixed into the elution buffer was removed and placed into a clean 2 ml tube without disturbing the beads. A rapid adaptor dilution was prepared and added to the barcoded DNA and left to incubate at room temperature while the flow cell (FLO-MIN114; version R10) was being prepared. A flow cell priming mix was prepared and used to prime the flow cell before loading the sample. A sequencing buffer (SB) and library beads (LIB) mixture from the kit was added to the barcoded DNA, creating a library. The library containing the barcoded DNA was loaded into the flow cell, being careful not to introduce any bubbles into the flow cell, as it can irreparably damage the cell. After the flow cell was loaded with the sample, it was inserted into the MinION MK1B device, and the sequencing workflow was set to run for 72 hours using the MinKNOW application (version 6.2.14).

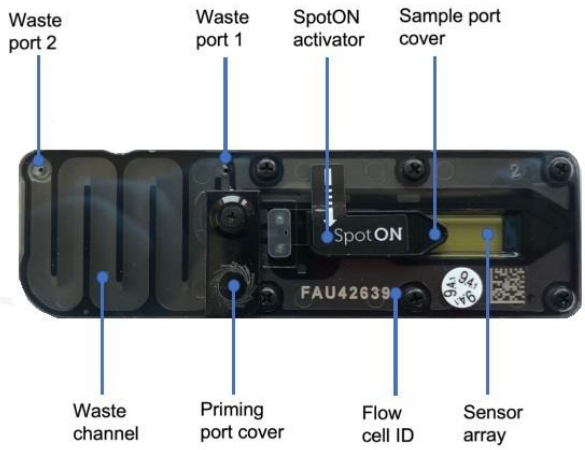


Figure S 3: A flow cell where the library sample is loaded via the SpotON port onto the sensor array, which contains the nanopores. The entire cell is inserted into the MinION device (Bautista *et al.,* 2023:16).

5.1.1.13 References

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5.1.2 Results

Table S1: Correlation table between microbial concentrations and physical parameters within the animal testing facility.

	Sampling method	Temperature (°C)	Relative Humidity (%)	CO_2 concentration (ppm)	CO concentration (ppm)	Air velocity (m/s)
Cloak room	Surface swabbing	r = -0.5 (p > 0.9)	r = 0.5 (p > 0.9)	r = 0.5 (p > 0.9)	Insufficient data	r = 0.8 (p = 0.6)
	Contact plate	r = -0.5 (p > 0.9)	r = 1.0 (p = 0.3)	r = 1.0 (p = 0.3)	Insufficient data	r = 0.8 (p = 0.6)
	Air sample	r = 0.5 (p > 0.9)	r = 1.0 (p = 0.3)	r = 1.0 (p = 0.3)	Insufficient data	r = 0.8 (p = 0.6)
Experimental room	Surface swabbing	r = 0.0 (p > 0.9)	r = 0.8 (p = 0.6)	r = 0.8 (p = 0.6)	r = 0.0 (p > 0.9)	r = -0.8 (p = 0.6)
	Contact plate	r = -0.5 (p > 0.9)	r = -1.0 (p = 0.3)	r = -0.5 (p > 0.9)	r = -0.5 (p > 0.9)	r = 1.0 (p = 0.3)
	Air sample	r = 1.0 (p = 0.3)	r = 0.5 (p >0.9)	r = -0.5 (p >0.9)	r = 1.0 (p = 0.3)	r = 0.5 (p >0.9)
Breeding room	Surface swabbing	r = 1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = -1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = -0.8 (p = 0.6)
	Contact plate	r = 1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = -1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = -0.8 (p = 0.6)
	Air sample	r = -0.5 (p > 0.9)	r = 0.5 (p > 0.9)	r = 0.5 (p > 0.9)	r = -1.0 (p = 0.3)	r = -0.8 (p = 0.6)
Washing area	Surface swabbing	r = 1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = 0.5 (p > 0.9)	r = 1.0 (p = 0.3)	r = -0.5 (p > 0.9)
	Contact plate	r = 0.5 (p > 0.9)	r = -0.5 (p >0.9)	r = 1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = 1.0 (p = 0.3)
	Air sample	r = 0.5 (p > 0.9)	r = -0.5 (p >0.9)	r = 1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = -1.0 (p = 0.3)
Clean hallway 1	Surface swabbing	r = -0.8 (p = 0.6)	r = -0.8 (p = 0.6)	r = -0.8 (p = 0.6)	r = 0.0 (p >0.9)	r = 0.8 (p = 0.6)
	Settle plate	r = 0.5 (p > 0.9)	r = 1.0 (p = 0.3)	r = 1.0 (p = 0.3)	r = -0.5 (p >0.9)	r = -0.5 (p >0.9)
Clean hallway 2	Surface swabbing	r = -0.8 (p = 0.6)	r = -0.8 (p = 0.6)	r = 0.0 (p >0.9)	r = 0.8 (p = 0.6)	r = 0.8 (p = 0.6)
	Settle plate	r = -0.5 (p >0.9)	r = -1.0 (p = 0.3)	r = -0.5 (p >0.9)	r = 1.0 (p = 0.3)	r = 0.5 (p > 0.9)
Dirty hallway	Surface swabbing	Insufficient data	Insufficient data	Insufficient data	Insufficient data	Insufficient data
	Settle plate	r = -1.0 (p = 0.3)	r = -1.0 (p = 0.3)	r = 0.5 (p > 0.9)	r = -0.5 (p > 0.9)	r = 0.5 (p > 0.9)

r presents the correlation coefficient, and the p-value indicates the statistical significance. Insufficient data result are due to no variations in one of the data sets leading to the inability to calculate the correlation coefficient. ppm = parts per million. m/s = meters per second.



Figure S 4: Dirty bedding swab sample growth on blood agar plates with alpha haemolysis.



Figure S 5: Microbial air sampler blood agar plate with two colonies with gamma haemolysis.

5.1.3 Supporting documents

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: http://www.nwu.ac.za

North-West University Health Research Ethics Committee (NWU-HREC)

Tel: 018 299-1206
Email: Ethics-HRECApply@nwu.ac.za (for human studies)

22 May 2024

RESEARCH ETHICS COMMITTEE LETTER OF DECISION: NO RISK

Based on the review by the North-West University Health Research Ethics Committee (NWU-HREC) on 22/05/2024, the NWU-HREC hereby clears your study as a no risk study. This implies that the NWU-HREC grants its permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: Hazardous biological agents in an animal testing facility and the occupational exposure risks
Principal Investigator/Study Supervisor/Researcher: Dr SR Horn
Student: L Lucas - 34921982

Ethics number: N W U - 0 0 0 9 7 - 2 4 - A 1
Institution Study Number Year Status

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study
Commencement date: 22/05/2024

Risk: No Risk

General conditions:

The following general terms and conditions will apply:

- *The commencement date indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-HREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research;*
  - *withdraw or postpone clearance if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the NWU-HREC or that information has been false or misrepresented;*
    - *submission of the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-HREC can be contacted for further information via Ethics-HRECApply@nwu.ac.za or 018-299 1206*

The NWU-HREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-HREC for any further enquiries or requests for assistance.

Yours sincerely,



_____

NWU-HREC Chairperson



_____

Head of the Faculty of Health Sciences Ethics Office for Research, Training and Support

Current details: (13210572) https://nwuac-my.sharepoint.com/personal/carolien_vanzyl_nwu_ac_za/Documents/NWU-HREC/NWU-HREC_App/HREC_App-2024/HREC_App05-20240612/NWU-00097-24-S1(SR%20Horn-L%20Lucas)-NR/NWU-00097-24-S1(SR%20Horn-L%20Lucas)-LoD/9.1.5.4.3_LOD_NWU-00096-24-A1_20240523.docm
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30 July 2025

LANGUAGE EDITING STATEMENT

I, Jannetje Levina De Kock hereby declare that the dissertation

Hazardous biological agents and the occupational exposure risks thereof in an animal testing facility

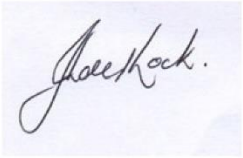
by

Lelani Lucas
34921982

in fulfilment of the requirements for the degree
Master of Health Science in Occupational Hygiene
at the North-West University,

- has been edited for language correctness and spelling.
- has been edited for consistency (repetition, long sentences, logical flow)

No changes have been made to the document's substance and structure (nature of academic content and argument in the discipline, chapter and section structure, and headings, order and balance of content, referencing style and quality).



J L DE KOCK

I did my best,
and God did the rest.

Hattie McDaniel