

Assessment and Reduction of Microbial Risks in Emergency Eyewash Stations

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Abstract

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This pilot study investigates the impact of regular and irregular flushing routines on microbial contamination in eyewash stations across a university, sampling a total of 70 eyewash stations. The primary objective was to assess whether regular flushing effectively reduces the risk of exposure to microbial contaminants, including Heterotrophic Plate Counts (HPC), *Legionella pneumophila*, and non-tuberculosis Mycobacterium. The results indicate that irregularly flushed eyewash stations showed a higher rate of unsafe microbial counts compared to those with regular flushing and maintenance at 99% confidence interval. *Legionella* was detected in a single building with irregular flushing, emphasizing the risk of such neglect. This study highlights the critical need for rigorous maintenance routines to ensure the safety and functionality of emergency eyewash stations. The findings advocate for regular flushing as a simple yet effective method to minimize potential health risks, reinforcing the importance of adherence to established safety standards in workplace environments. This pilot study lays the groundwork for future research, suggesting further investigation into the specific conditions that influence microbial presence and the development of maintenance strategies for varying conditions.

Dedication

To my family,

To my parents, *Assaad Assaad* and *Fatme Chaachoua*, for their support and love. Your endless sacrifices and belief in me have shaped me into the person I am today. I am forever grateful for your guidance, wisdom, and unwavering love.

To my husband, *Hadi Ayache*, for his constant encouragement, understanding, and patience have been my source of strength during the challenging moments of thesis writing. This accomplishment is not mine alone, but a testament to the strength we've built together. Thank you for always inspiring me to reach greater heights.

To my siblings, *Mohamad, Ali*, and *Narjes*, for their belief in me and for always having my back.

To my sister-in-law, *Ghofran*, and my niece, *Fatouma*, thank you for filling our family with joy, laughter, and new beginnings.

This thesis is dedicated to each one of you, my guiding lights, and sources of inspiration.

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Chapter I: Introduction

This study investigates the impact of maintenance routines on the microbial safety of emergency eyewash stations at the University of Washington, highlighting the potential health risks associated with irregularly flushed stations. By evaluating microbial counts and the presence of *Legionella pneumophila* and non-tuberculous mycobacteria, we underline the importance of regularly flushing and maintaining eyewash units. The primary problem addressed by this research is the increased risk of microbial contamination from inadequate eyewash maintenance practices, which can pose serious health threats to workers who are exposed to harmful substances or environments in workplaces. This study aims to mitigate these risks by presenting data collected from various emergency eyewash stations, proposing improvements to maintenance routines.

1.1 Overview of Workplace Eyewash Stations

Eye injuries in the workplace are alarmingly common; the National Institute for Occupational Safety and Health (NIOSH) reports that every day about 2,000 U.S. workers sustain job-related eye injuries requiring medical treatment (NIOSH, 2006). Nearly 20,000 incidents reported annually across the United States lead to at least one day away from work, according to the U.S. Bureau of Labor Statistics (U.S. Bureau of labor Statistics, 2023). Around 60% of these injuries result from workers rubbing their eyes or due to foreign bodies in the eye (U.S. Bureau of labor Statistics, 2023).

In workplaces where hazardous materials are commonly used, keeping workers safe is important. Emergency eyewash stations are a key safety tool providing rapid relief if workers are exposed to dangerous substances, such as harsh chemicals, solvents, other industrial compounds, as well as small debris that might get into the eye. Emergency eyewash stations are used immediately after exposure to these substances to flush out the eyes with clean water. This quick response is critical to curtail injuries, thwart chemical burns, or reduce the risk of long-term damage or blindness. These stations are specifically important for protecting the eyes, but their effectiveness depends on prompt use and proper maintenance. Many eyewash stations remain unused for extended periods, which results in a significant buildup of microbial contaminants

within the system's pipes and connections, posing a potential health and safety hazard to workers.

According to the American National Standards Institute/International Safety Equipment Association (ANSI/ISEA) Z358.1-2014 standard, eyewash stations must be easy to see, reach, and operate effectively to provide immediate relief for workers following their exposure to harmful chemicals. These workers may include laboratory students and researchers, manufacturing workers, first responders, and others who work in industries or environments where eye injuries or exposure to hazardous materials may occur.

The implementation of these guidelines is essential and has been advocated by various organizations beyond ANSI/ISEA. For instance, the Occupational Safety and Health Administration (OSHA) in its regulation 29 CFR 1910.151 Medical Services and First Aid mandate requires employers to equip their facilities in accordance with the widely recognized ANSI Z358.1 standard. This standard is a fundamental part of safety codes and is included in the International Plumbing Code (International Plumbing Code, 2021; OSHA, 2005).

As such, the focus on eyewash stations in this study stems from their serious role in emergency relief and their susceptibility to microbial contamination due to infrequent use and irregular flushing, which can lead to stagnant water that fosters the growth of biofilms and harmful microbes. The ANSI standard requires that eyewash stations be activated weekly for at least three minutes to flush out sediments and bacterial buildup effectively. This regular activation is not only a compliance measure but a practical necessity to clear supply lines of sediments and bacteria buildup resulting from stagnant water.

1.2 Importance of Eyewash Stations Safety

Standards from ANSI/ISEA and OSHA form the basis of our discussion on the essential features and maintenance routines for eyewash stations. A key requirement of those guidelines is accessibility, where the standard specifies the placement and accessibility of emergency eyewash and shower equipment. The underlining of accessibility comes to ensure eyewash stations are accessible in emergencies: a station must be within a 10-second walk from hazardous locations to accommodate workers who might be partially blinded. The station should remain unobstructed and clear of any items that could delay its use, with nozzle covers that are airtight to prevent clogs from contaminants or debris. These covers must be easily removable by water flow,

enabling operation by individuals with limited vision (Kelechava, 2018; OSHA Archive, 1992). In addition to accessibility, the Z358.1-2014 standard also emphasizes the durability and reliability of eyewash units. The guidelines state that eyewash units should be made of substances that will not corrode when in contact with water. As part of the design standards, the eyewash unit should provide a controlled flow to both eyes simultaneously to ensure proper flushing; this design would maximize the chances of complete decontamination. In addition, the temperature of the flushing fluid should be moderate, ideally between 16 and 38 degrees Celsius. This is to avoid any discomfort that might discourage a full 15-minute flush, which is necessary for many contaminants. Additionally, an annual inspection is required to check for compliance with safety regulations. By adhering to these standards and protocols, workplaces can reduce the severity of eye injuries and infections due to contaminated flushing fluid and ensure quick and effective response in case of an emergency (Kelechava, 2018; OSHA Archive, 1992).

While avoiding exposure is always the first line of protection, accidental exposure to dangerous chemicals can still occur. The first 10 to 15 seconds after contact with a hazardous, especially corrosive, substance are crucial and any delay in administering immediate treatment can lead to severe eye injuries (Canadian Centre for Occupational Health and Safety [CCOHS], 2024). This is why the accessibility, durability, and design of eyewash stations are critical factors in effectively mitigating or exacerbating emergency situations.

According to OSHA, when eyewash stations fall short of maintenance, they may harbor microorganisms that can enter the eye or skin, or even be inhaled during use. Workers with skin cuts or weakened immune systems, perhaps from conditions like transplants, cancer, or autoimmune diseases, face a higher risk of adverse effects from any microbial presence in the water. Employees using an eyewash station that hasn't been properly maintained might start noticing symptoms like irritation or swelling of the eyes, increased tearing, difficulty with vision, sensitivity to light, and general discomfort in the eyes. These symptoms could take a few days to appear after being exposed to water from an eyewash station that contains contaminants (OSHA, 2015). Microorganisms like *Acanthamoeba*, *Pseudomonas*, and *Legionella* specifically, can have serious potential to cause severe infections (OSHA, 2015). With these risks in mind, we now delve into the specific microbial risks associated with poorly maintained eyewash stations.

1.3 Risks Associated with Microbial Contamination

This research targets microbial count, *Legionella pneumophila*, non-tuberculosis mycobacterium species like *Mycobacterium chelonae* and *Mycobacterium avium* complex, and *Acanthamoeba* species. These organisms were previously detected in anthropogenic water systems and pose significant health risks (Paszko-Kolva et al., 1991; Vantarakis et al., 1998). *Legionella pneumophila* and non-tuberculosis mycobacteria can cause lung infections from inhaled water droplets, with non-tuberculosis mycobacteria also leading to a range of ocular infections including keratitis, while *Acanthamoeba* can cause severe eye infections (Bai et al., 2023; Das et al., 2024; Fanselow et al., 2021; Ratnatunga et al., 2020). Furthermore, there exists a parasitic interaction where *Legionella pneumophila* can reproduce inside the *Acanthamoeba* (Dietersdorfer et al., 2016). Understanding these associations is essential in assessing the potential risks present in eyewash stations and highlights the need for strict maintenance practices to ensure worker safety in the event of an emergency. In this study, to measure the microbial presence, heterotrophic plate counts (HPC) were tested, which offers a general indication of the microbial load in the water sample.

1.3.1 Heterotrophic Plate Count: Importance of Heterotrophic Bacteria in Water Quality Assessment

In assessing water quality, particularly in the context of eyewash stations, studies have shown that Heterotrophic Plate Counts (HPC) serve as an important measure. HPC tests quantify the levels of heterotrophic bacteria, which include a wide variety of organisms present in water; these bacteria are not necessarily harmful but can indicate the overall microbial state of the water. The Center for Disease Control and Prevention (CDC) recommends that HPC levels in potable water should ideally remain below 500 Colony Forming Units per milliliter (CFU/mL) to maintain good water quality. Elevated HPC levels that are consistently above this threshold, suggest potential stagnation or deterioration in water quality and signals a need for maintenance or treatment interventions. While HPC is an indicator of water condition, it is important to note that it does not directly measure pathogenic bacteria such as *Legionella*. Thus, a lower HPC count, while indicative of fewer heterotrophic bacteria, does not necessarily confirm the absence of all harmful microbes. To effectively manage microbial content, including pathogens, in water systems, additional specific tests are required. HPC testing, therefore, complements these tests by

providing a broader overview of water quality and helping to identify when more in-depth analyses is necessary (CDC, 2015; LeChevallier et al., 1987)

Regular application of HPC testing is fundamental in maintaining the safety of water systems, as it covers a broad spectrum of microorganisms that thrive in nutrient-rich environments under suitable growth conditions (Dehghani et al., 2011). A study from 1989 underscores the importance of routine HPC monitoring in both stationary and portable eyewash units ([Figure 1.1](#) shows those two different types of eyewash units). This study suggests that infrequent flushing could lead to a rise in heterotrophic bacteria organisms that could pose a safety concern if used for eye decontamination. Without consistent flushing, biofilm formation along the interior surfaces of the eyewash stations becomes a considerable risk. Biofilms act as reservoirs for bacteria that thrive and grow if left on their own for extended periods.

In 2023, Swanson et al. conducted a study to assess the effects of water stagnation on microbial growth in eyewash stations. Using a detailed protocol, they monitored multiple stationary eyewash stations at a facility where they allowed water to stagnate for one to seven days. The study found increased microbial contamination correlated with longer stagnation periods, as evidenced by rising Heterotrophic Plate Counts (HPC). Furthermore, the research demonstrated that flushing eyewash stations for prolonged periods significantly reduced HPC levels. Specifically, a 10-minute flush dramatically reduced microbial counts, while a 20-minute flush almost eliminated all bacterial presence. These findings emphasize the importance of flushing for extended periods for safer eyewashes. (Swanson et al., 2023).

Figure 1.1 *Stationary (Plumbed) and Portable Eyewash Stations*



Source: 1 <https://trdsf.com/blogs/news/plumbed-vs-portable-eye-wash-station>

1.3.2 Waterborne Pathogens: Legionella

As mentioned earlier, *Legionella* bacterium, one of the bacteria that can cause serious respiratory illnesses, such as Legionnaires' disease, when inhaled from water droplets (Hamilton et al., 2018). This bacterium is characterized by being small, gram-negative rod-shaped without a capsule or spores and ranging in size, generally being narrow and varying in length (Winn, 1988). This bacterium is distinguished by its mobility, facilitated by flagella, and possesses cell walls that are notably different from other gram-negative bacteria due to the presence of branched-chain fatty acids (Campbell et al., 1984). *Legionella*'s metabolism is aerobic and microaerophilic, relying on amino acids rather than fermentative processes for energy and carbon (Campbell et al., 1984). It can withstand a range of acidity, and oxygen levels, making it highly adaptable to different aquatic habitats (Nguyen et al., 1991). It is also known for its resilience across a broad spectrum of temperatures, ranging from 16.5°C to 64°C, with some strains even withstanding hotter temperatures in both natural and artificial environments (Bentham, 1993; Botzenhart et al., 1986; Henke & Seidel, 1986).

Legionella is a significant concern in the world of waterborne pathogen as it thrives in water conditions found in a variety of environments ranging from natural freshwater bodies like rivers, lakes, and ponds to the artificial water systems of buildings, including those in eyewash stations (Fields, 1996). It grows well in these environments because it can form protective biofilms and it also has resistance to some disinfection methods allowing it to persist and multiply even in treated water systems (Marin et al., 2022). Without proper maintenance, the use of these units may form dangerous aerosols that carry the bacteria (OSHA, 2015).

While not a cause of eye infections, *Legionella* infections largely occur through inhalation, typically manifesting as one of two conditions: the flu-like Pontiac fever or Legionnaires' disease (US EPA, 2016). Legionnaires' is a more serious condition and can be fatal (OSHA, 2015). Those particularly vulnerable to *Legionella* include individuals with weakened immune systems, people older than 55, and those with existing respiratory conditions like Chronic Obstructive Pulmonary Disease (COPD) (OSHA, 2015). Symptoms like coughing, shortness of breath, high fevers, muscle pains, and headaches usually appear within 2 to 14 days after exposure and might require hospitalization (OSHA, 2015). Because routine environmental monitoring for *Legionella* is not typically conducted, its presence is often revealed only in case of illness outbreaks. Therefore, maintaining and regularly disinfecting water systems, especially

in places prone to bacterial growth like eyewash stations, is crucial for early detection and prevention of disease.

This bacterium also cohabitates with amoebae, using them as hosts or as an amplifying agent for growth, thereby persisting and spreading across various aquatic ecosystems through symbiotic relationships with at least 13 different amoebae species (Fields, 1996; Henke & Seidel, 1986; Kramer & Ford, 1994; Lee & West, 1991; Paszko-Kolva et al., 1991, 1993; Vandenesch et al., 1990).

1.3.3 Waterborne Pathogens: Non-Tuberculosis Mycobacterium

Nontuberculous mycobacteria (NTM) are abundant environmental pathogens that are commonly found in soil, dust, and water, including both natural water sources such as rivers and lakes, and municipal water sources used for drinking and showering. NTM is known to form biofilms that can adhere to surfaces in moist environments, like the insides of plumbing systems in buildings. Biofilms serve as a preferred habitat for NTM because the bacteria's cell walls, which repel water, enable them to attach quickly to surfaces and multiply, even in environments with minimal nutrients (CDC, 2019; Norton et al., 2004).

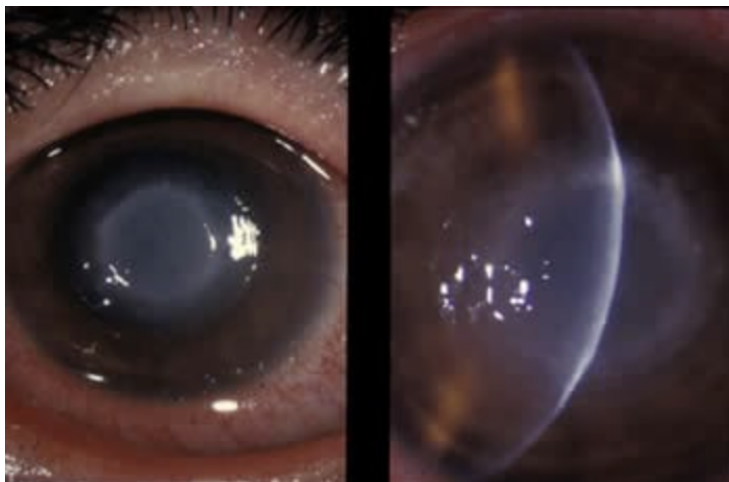
Occurrences have shown that drinking water systems can sometimes be identified as the source for NTM infections, like *M. avium* or *M. chelonae* (Loret & Dumoutier, 2019). This was confirmed by matching the bacteria from clinical cases to those found in water sources using biochemical tests and/or molecular methods (Falkinham, 2015; Falkinham et al., 2008; Loret & Dumoutier, 2019; Regnier et al., 2009; von Reyn et al., 1994). Contact with, inhalation, or ingestion of water containing atypical *Mycobacterium* spp. can lead to a range of illnesses affecting various body systems including lung, blood, skin, or eye infections. Consequently, controlling the presence of NTM in water systems is a significant preventive measure against these infections (Loret & Dumoutier, 2019).

1.3.4 Amoebae and Water Quality

Species of free-living amoebae like *Hartmannella*, *Vahlkampfia*, and *Acanthamoeba* have been discovered and isolated in eyewash stations (OSHA, 2015). *Acanthamoeba* is a microscopic single cell organism (amoeba) that may cause eye infections such as *Acanthamoeba* keratitis (see [Figure 1.2](#) to visualize *Acanthamoeba* infection in the early stages within the epithelial layer of

the eye). Detecting *Acanthamoeba* due to an infection can be particularly difficult, because its symptoms closely mimic those of any other eye conditions, such as eye redness, pain, tearing, blurred vision, light sensitivity, and eye inflammation occurring several days after using a contaminated eyewash station. (OSHA, 2015). Stationary and portable eyewash stations, when left unused for long periods of time, can become reservoirs for amoebic organisms. It is important to note that *Acanthamoeba* spp. infections tend to show a high level of resistance to treatments with antibiotics (Paszko-Kolva et al., 1991). This organism can survive in treated water and is often found in mucous membranes, such as the nose, throat, and eyes, as well as in neurological tissue, such as the brain, typically without causing any harm to the person (OSHA, 2015). Individuals with weakened immune systems are at a higher risk of developing serious infections, neurological or systemic infections, like Granulomatous Amoebic Encephalitis (OSHA, 2015). Eye symptoms such as pain, tearing, redness and inflammation may be experienced by workers after exposure to a contaminated eyewash station. These symptoms often appear days post-exposure.

Figure 1.2 *Eye Infection from Acanthamoeba*



Source: 2 <https://www.cdc.gov/contactlenses/parasitic-keratitis.html>

The presence of free-living amoebae, notably *Acanthamoeba* and *Hartmannella*, presents a significant risk, particularly for soft contact lens wearers, due to potential infections resulting from improper hygiene practices and contact with contaminated water (CDC, 2019; NIYYATI et al., 2014). A study done in 1987, investigated that such a presence in eye wash stations can cause

severe infections especially when introduced into traumatized eyes, which may include those of workers using eyewashes due to certain exposures. Additionally, measures such as flushing stationary eye wash stations and treating portable stations with 25 ppm of free chlorine have been shown to reduce the number of stations harboring amoebae. These findings highlight potential strategies for controlling microbial contamination in eyewash stations (Tyndall et al., 1987).

Recognizing the importance of these emergency facilities, we next outline the specific aims that will direct the focus of our study.

1.4 Specific Aims

1.4.1 Specific Aim 1: Quantify Heterotrophic Bacteria Levels

Quantify the level of heterotrophic bacteria through Heterotrophic Plate Counts (HPC) in workplace eyewashes and compare the growth on plates between regularly and irregularly flushed samples.

1.4.2 Specific Aim 2: Evaluate Presence of Pathogenic Microorganisms

Evaluate the presence or absence of *Legionella pneumophila*, Non-tuberculosis Mycobacterium, and *Acanthamoeba* in workplace eyewash stations.

1.4.3 Specific Aim 3: Estimate Effectiveness of Flushing Routines

Estimate the effectiveness of both flushing routines.

Chapter II: Methods

2.1 Study Design and Sampling Strategy

2.1.1 Sampling Procedure and Protocol

This pilot study employed convenience sampling of water from 70 emergency eyewash stations across 21 buildings from the University of Washington. Sampling activities were conducted from September 12th, 2023, through January 29th, 2024. Outreach documents were prepared and distributed to individuals responsible for overseeing the designated spaces. These documents requested their participation and secured permissions to collect samples from the eyewash stations, as detailed in [Appendix B](#).

The types of buildings sampled included research laboratories, teaching facilities (such as classrooms with teaching labs), shared areas (particularly hallways with eyewash stations), and specialized shops like fabrication shops available for student use. These buildings vary in age, constructed between 1908 and 1998, and are actively used by different departments, schools, and for various purposes including research, teaching, and workshops. Regarding maintenance, the flushing schedules of eyewash stations varied significantly, even within the same building. The flushing routines were categorized into two types based on the information provided by lab personnel or found on log sheets, each reflecting different levels of compliance and maintenance, which could influence the findings related to water quality and safety compliance:

- Regularly Flushed: These stations are reportedly flushed weekly according to records or personnel claims. However, the consistency and adequacy of these flushes in terms of duration remain uncertain. It is claimed that these stations have been flushed within the last 7 days.
- Irregularly Flushed: Stations in this category are not flushed on a weekly basis. Many lack complete flushing records and are frequently reported by facility managers as being poorly maintained or associated with inactive lab spaces.

On the day of sampling, a cooler with sterilized bottles was prepared for collecting the samples. Gloves were also brought along to ensure sterility and minimize contamination during the process, especially in specific lab environments. Bottles intended for sample collection were pre-labeled with essential details such as the date and location of collection before leaving the

laboratory. This method helped guarantee accurate labeling, facilitating traceability and data reliability throughout the sampling process.

Each sample, containing 1 liter of water, was collected immediately upon activation of the eyewash, ensuring collection from the initial discharge. Sterile plastic bottles were used to prevent contamination, with collection initiated promptly following eyewash activation. Throughout the sampling procedure, water was collected immediately prior to its contact with anything. Measures were implemented to manage any spills or splashes from the eyewash stations due to water collection, with rapid resolution of any such incidents through containment and disposal of water. Upon completion of sampling, the laboratory environment was returned to a clean state, as it was before.

2.1.2 Data Recording and Storage

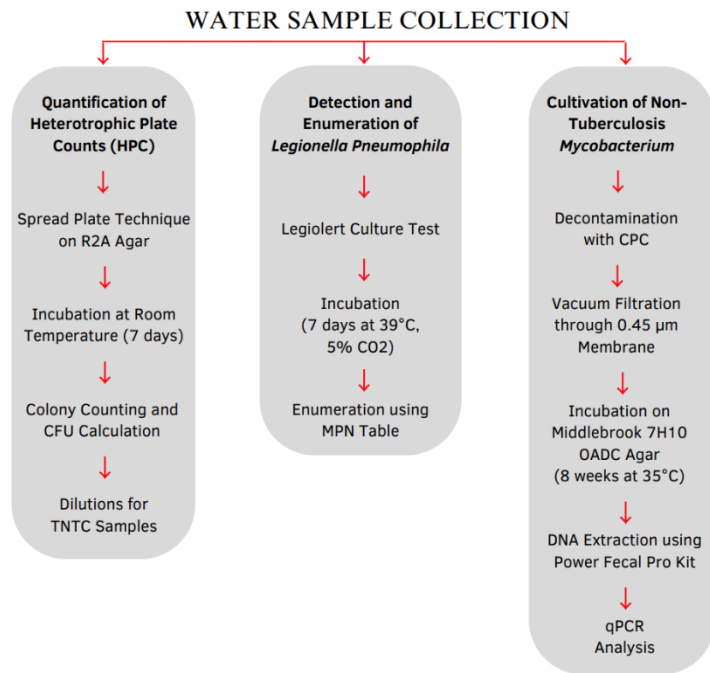
The flushing frequency of all eyewashes was documented based on available records, and each sample was accurately labeled with the flushing frequency category, date, and location of collection. To preserve the samples during transport, all samples were stored in a cooler and subsequently transferred to a refrigerator at 4°C within 2 hours of collection.

Following collection and storage, the samples were processed within a 48-hour timeframe. Upon return to the EOHML Laboratory, all collected data, including sample numbers and locations, were logged into an Excel spreadsheet (refer to [Appendix A](#)). This spreadsheet served as a source for tracking essential information, including Sample ID, collection dates, sample processing dates, flushing routines, and any relevant observations gathered from the field or environmental settings where the eyewash samples were collected.

2.2 Analysis of Water Samples

In this section, we outline the methods used in the study to assess the microbial quality of water from eyewash stations. The methods are summarized in [Figure 2.2](#), which provides a visual guide to the procedures followed. These methods include Heterotrophic Plate Counts (HPC) for evaluating overall bacterial load, as well as specific tests for detecting pathogens such as *Legionella* and non-tuberculosis Mycobacterium. Each method is described in detail in subsequent subsections, explaining the steps through data analysis.

Figure 2.2 Schematic Diagram of Methods

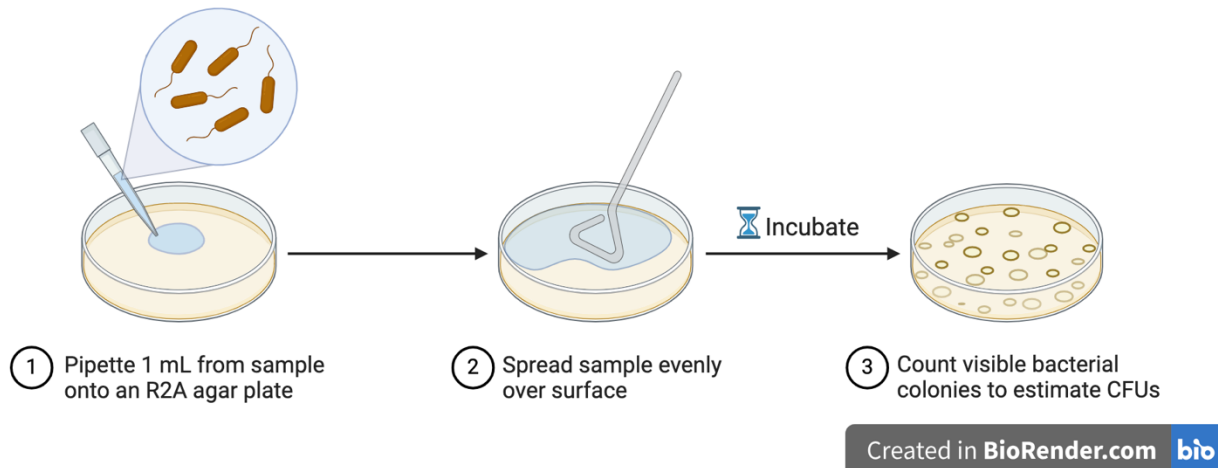


2.2.1 Quantification of Heterotrophic Plate Counts (HPC)

The HPC analysis involved the utilization of the spread plate technique on R2A agar, a well-established method in microbiology for quantifying bacterial populations (Paszko-Kolva et al., 1991). This agar medium was chosen for its selectivity in supporting the growth of heterotrophic bacteria present in environmental samples, particularly water (Paszko-Kolva et al., 1991).

To ensure each sample was homogeneous and representative, it was inverted five times before a 1 mL aliquot was aseptically transferred onto a 105 mm R2A agar plate. The sample was evenly spread using disposable cell spreaders to ensure uniformity. The agar plates were then incubated upside down at room temperature for 7 days to promote optimal bacterial growth. After incubation, each plate was inspected for any irregular growth, and the visible bacterial colonies were counted to estimate the colony-forming units (CFU) per milliliter of water. This quantification process, as depicted in [Figure 2.3](#) below, demonstrates the steps taken to assess bacterial spread using the heterotrophic plate count method.

Figure 2.3 *Quantification of Heterotrophic Plate Count Method*



When the number of Colony Forming Units (CFU) per plate exceeded 250, plates were recorded as Too Numerous to Count (TNTC). For samples with TNTC colonies, 10-fold and 100-fold dilutions were performed using Phosphate-Buffered Saline (PBS) as the diluent (Simpson et al., 2022). TNTC plates indicated that the original sample had a colony count exceeding the countable range. Dilutions were necessary to bring the colony count within a manageable range for accurate counting. A 10-fold dilution involved taking 1 mL of the original sample and diluting it in 9 mL of PBS. This process was repeated to create a 100-fold dilution by taking 1 mL from the 10-fold dilution and diluting it in 9 mL of PBS.

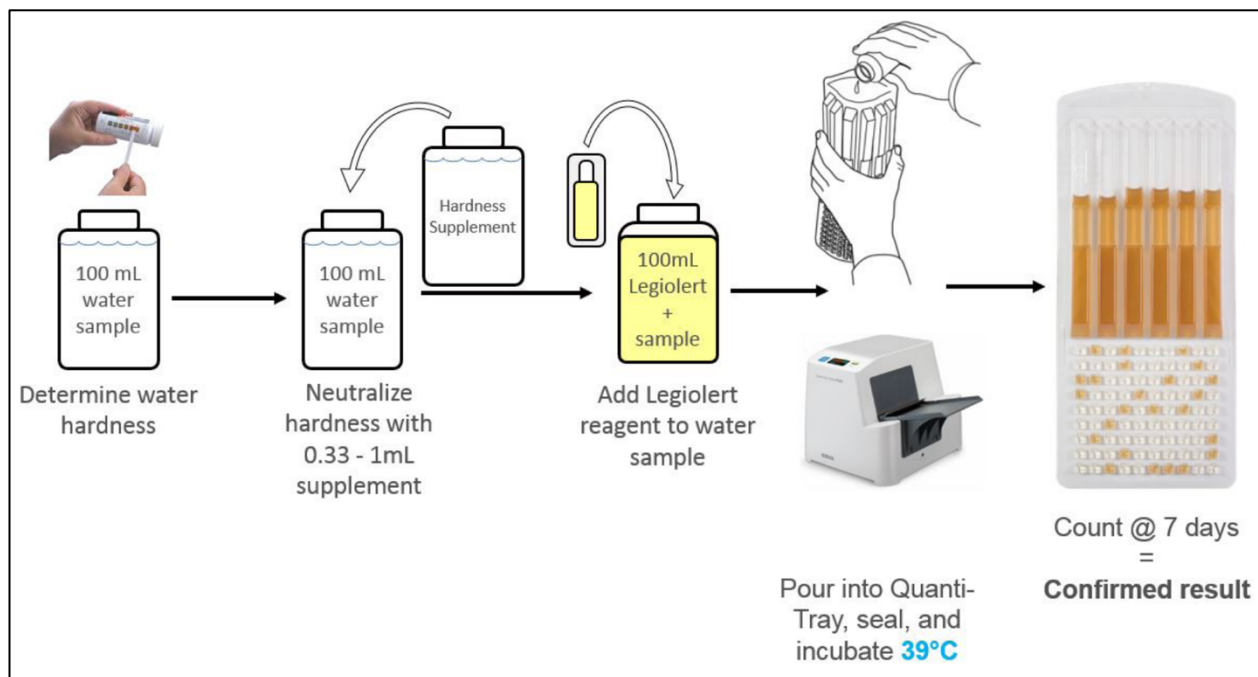
2.2.2 *Detection and Enumeration of Legionella Utilizing the Legiolert Kit*

In this study, the detection of *Legionella pneumophila* was conducted using the IDEXX Legiolert[®] culture test, following the 100 mL potable water protocol (IDEXX Laboratories, 2023). According to the protocol, 100 mL aliquots of each water sample were first brought to room temperature in sterile bottles and assessed for water hardness using test strips to determine the appropriate supplement dosage. The test strips for all our samples indicated that all samples were classified as soft water. Consequently, 330 μ L of the reconstituted Legiolert[®] supplement was added to each sample.

Next, the contents of one Legiolert[®] blister pack were added to each sample aliquot and thoroughly mixed by agitation until fully dissolved. Subsequently, the prepared samples were dispensed into Legiolert[®] trays and securely sealed using a Quanti-Tray[®] sealer PLUS. The

sealed trays were then incubated paper side down for a duration of 7 days at a temperature of 39°C within an incubator environment humidified with autoclaved DI water and maintained at a 5% CO₂ atmosphere. Following the incubation period, any brown or turbid wells observed in the Legiolert[®] trays were carefully noted and utilized to determine the presence of *Legionella pneumophila* using the Legiolert Most Probable Number (MPN) Table (refer to [Appendix B](#)) (IDEXX Laboratories, “n.d.”). Reports were provided in MPN, equivalent to CFU per ISO 6107:2021, ensuring compliance with international standards for microbial enumeration in water samples (Checa et al., 2021). The process of performing analysis for *Legionella pneumophila* using a Legiolert kit is illustrated in [Figure 2.4](#), which was adapted from a study titled *Managing Legionella pneumophila in Water Systems* (LeChevallier, 2020).

Figure 2.4 Overview of the Legiolert[®] Test Potable Water 100 mL Protocol



2.2.3 Cultivation of Non-Tuberculosis Mycobacterium

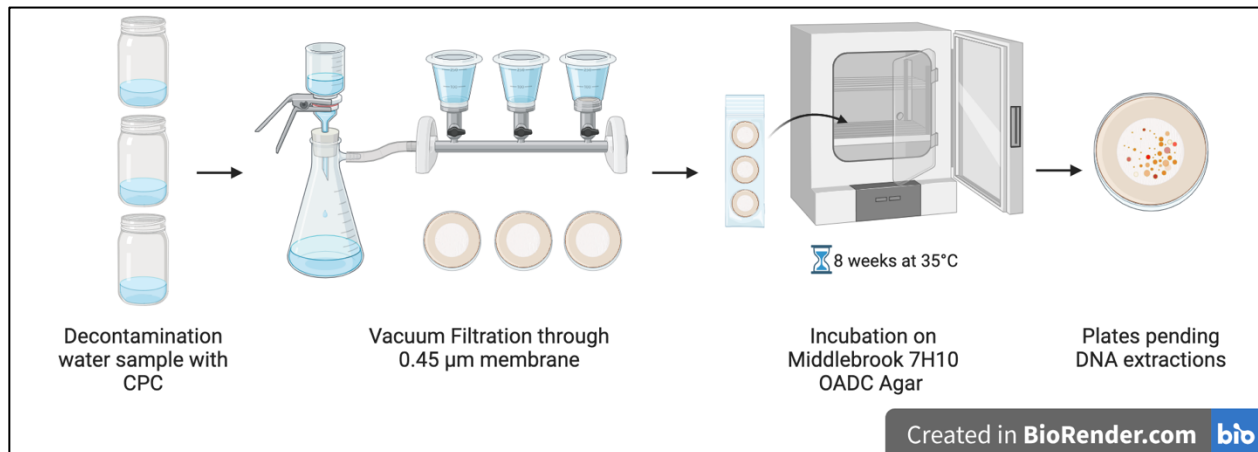
The targeted Non-tuberculosis Mycobacterium species for this study are *Mycobacterium chelonae* and *Mycobacterium avium* complex (MAC). The selection of these species results from their potential to cause respiratory infections from aerosolized water particles. Initially in the processing stage, 200 mL of each sample was precisely aliquoted into sterile bottles containing

15 mg of cetylpyridinium chloride (CPC) for decontamination purposes. The inclusion of CPC, known for its strong antimicrobial properties, aimed to eliminate all organisms except mycobacterium due to their robust nature. The samples were manually mixed and allowed to stand undisturbed for 30 minutes to facilitate the decontamination process. Following decontamination, the samples underwent vacuum filtration through a 0.45 µm pore-size membrane. Upon completion of filtration, the filters underwent a thorough rinsing process with 300 mL of autoclaved DI water. It's important to note that during our initial methods development, attempts to use DI water directly from the tap resulted in severe contamination, requiring us to autoclave all the DI water used in subsequent sample processing steps.

Subsequently, the membrane filters were aseptically transferred to 60 mm plates containing Middlebrook 7H10 OADC agar, where they were incubated inverted at a controlled temperature of 35°C for a duration of 8 weeks. For a visual representation of the plating method for non-tuberculosis Mycobacterium on 7H10 OADC agar, please refer to [Figure 2.5](#) below. To prevent agar dehydration, the plates were carefully enclosed in plastic bags throughout the incubation period. Weekly examinations and photographic documentation were conducted to monitor microbial growth and development on the plates during the monitoring process. During the first week, examinations were performed every two days to closely observe any early signs of microbial proliferation. Afterward, the monitoring frequency transitioned to weekly intervals for the remaining 7-week duration of the incubation period.

Following the incubation period, the plates were stored at 4°C pending DNA extraction procedures. DNA extractions from the samples were performed using the Power Fecal Pro kit on the QiaCube Connect platform, which is an automated system used for DNA extractions. Consequently, quantitative PCR (qPCR) analysis was conducted to determine the presence of genomic DNA from Mycobacterium species within each sample. The detailed procedure of qPCR analysis is explained in [Section 2.3](#) for a comprehensive understanding of the analytical process. It is important to note that, due to the long incubation period of 8 weeks required for Mycobacterium and the temporary discontinuation of the project for several months due to administrative processes, DNA extraction and qPCR analyses were conducted on only 38 of the 70 collected samples.

Figure 2.5 *Non-Tuberculosis Mycobacterium* Plating Method Overview



2.3 DNA Extraction from Plates

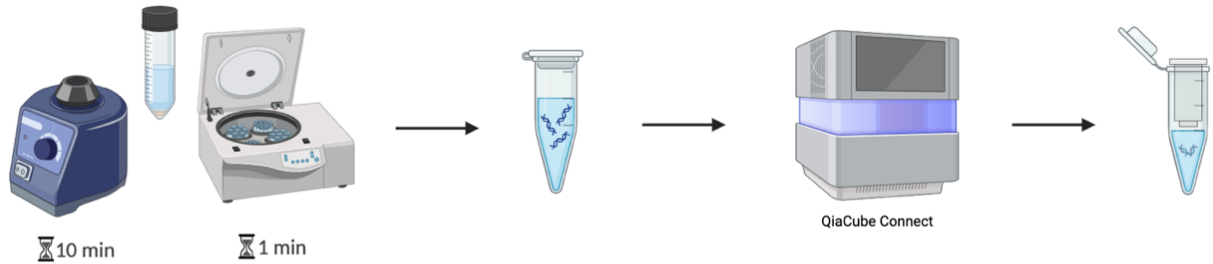
Following the incubation period of 8 weeks, DNA extraction was performed from individual colonies grown on each plate. Subsequently, Quantitative PCR (qPCR) was used to determine the *Mycobacterium* species present on the plates. The DNA extraction process was carried out using the Power Fecal Pro kit, which is optimized for extracting DNA from microbial samples, on the QiaCube Connect platform (Qiagen, 2013). The QiaCube Connect is an automated system designed to streamline and standardize DNA extraction procedures, ensuring reproducibility and efficiency. Upon completion, the DNA extractions were stored at -20°C in the laboratory freezer for subsequent analysis. For a detailed illustration of the extraction and qPCR process, please refer to [Figure 2.6](#).

2.4 Quantitative Polymerase Chain Reaction (qPCR)

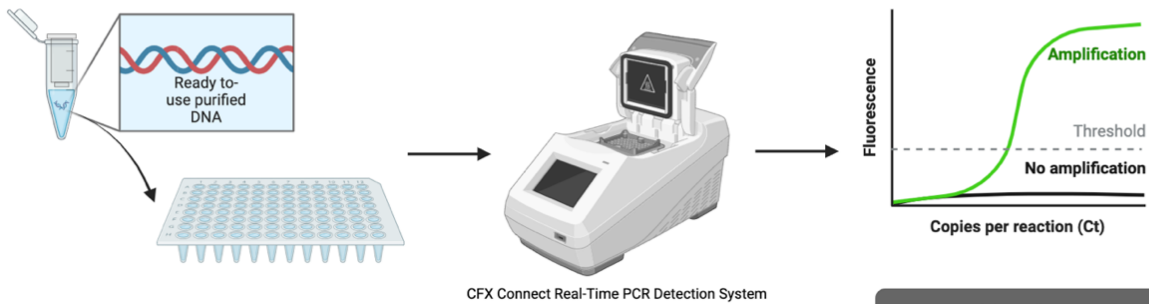
To ensure the reliability and accuracy of qPCR results specific to *Mycobacterium avium* complex (MAC) and *Mycobacterium chelonae*, appropriate controls were incorporated into the experimental protocol. Negative controls were included to monitor for contamination, while *Mycobacterium chelonae* served as a positive control which was used to validate the sensitivity and specificity. Throughout the qPCR analysis, control samples were processed alongside experimental samples to mitigate any potential variability in the procedure. These control samples provided critical benchmarks, allowing us to assess the reliability and accuracy of our qPCR assay and attribute any observed results to the target DNA sequences present in the experimental samples.

Figure 2.6 Process Flowchart: DNA Extraction and qPCR Analysis

Step 1:
DNA extraction



Step 2:
qPCR Analysis



Created in **BioRender.com** **bio**

The primary objective of the qPCR analysis was to detect the presence of genomic DNA from *Mycobacterium* species within the extracted colonies. qPCR analysis was performed using the CFX Connect Real-Time PCR Detection System, an instrument capable of rapid and precise quantification of DNA targets (CFX Connect Real-Time PCR Detection System | Bio-Rad, 2024). For the qPCR reaction, previously described primers and probe were reported in an NIH published article that targets both mycobacterium strains this study is looking for, *Mycobacterium chelonae* and *Mycobacterium avium* (Radomski et al., 2013). qPCR amplification was performed using forward primer FatpE, reverse primer RatpE, and probe PatpE in duplicate assays (Radomski et al., 2013). [Table 2.2](#) below gives information on the primers and probe used. For a comprehensive overview and detailed breakdown of the qPCR experimental setup, refer to [Appendix B](#).

Table 2.2 *Primer Sequence for Mycobacterium qPCR*

	Primer Sequences (5 → 3)	Orientation	Amplicon Tm°C
FatpE	CGGYGCCGGTATCGGYGA	Forward	62°C
RatpE	CGAAGACGAACARSGCCAT	Reverse	59°C
Probe PatpE	ACSGTGATGAAGAACGGBGTRAA		68°C

2.5 Data Analysis Using R Studio

The analysis of the results was conducted using R Studio, utilizing several R packages to reorganize data visualization and statistical testing. “**readxl**” package was used to import data from Excel files. “**tidyverse**” and “**dplyr**” packages were used to facilitate data summarization. To analyze the occurrence and distribution of HPC bacteria, the Mann-Whitney U test, also known as the Wilcoxon rank-sum test, was performed using the “**wilcox.test**” function in the “**stats**” module. This non-parametric test was chosen to compare differences between two independent groups when the assumption of normality is not met. Additionally, Fisher’s Exact Test will be used to examine the association between flushing routines and the presence of Mycobacterium species.

Chapter III: Results

3.1 Overview of Study Population and Sampling Locations

3.1.1 Observations of Lab Space and Eyewash Station Accessibility

Eyewash stations exhibited a range of conditions. While some were well-maintained, others were clearly outdated or in poor condition. The design of some eyewash stations appeared outdated, suggesting a need for updates to newer, more practical models. Issues with eyewash caps were common; many were either broken or did not close properly, leaving the stations unprotected from debris and making them susceptible to bacterial contamination. Some stations failed to meet hands-free operational standards; upon activation, the caps did not automatically remove due to the water pressure, thus violating safety regulations that require eyewashes to be hands-free and immediately operational. Also, debris found inside the eyewashes upon the removal of protective caps further indicated a lack of regular cleaning and maintenance even in allegedly maintained eyewashes.

3.1.2 Observations of Water Quality in Sampled Eye Wash Stations

Upon activating the eyewash stations and collecting the samples, some water samples immediately appeared unsuitable for use. The transparent to white color of the collection bottles made it easy to observe the water's condition. In several instances, the water emitted an unpleasant odor and appeared turbid. The color of the water collected varied, with some samples showing an orange to yellowish shade. Photographs of these water samples, demonstrating the range of colors and sediment accumulations, are included in the [Appendix A](#) for reference. Several bottles contained visible sediments settling at the bottom, ranging in color from black and orange to red and rust-like tones. In other samples, small black debris was noted.

3.2 Heterotrophic Plate Counts (HPC) Analysis

3.2.1 Results of HPC Quantification for Each Sampled Eye Wash Station

This section presents the results of Heterotrophic Plate Counts (HPC) performed on the samples from various eyewash stations. [Table A.1](#) in [Appendix A](#) summarizes the HPC results for each of the 70 eyewash stations sampled, categorized by location and flushing frequency. In addition to the results summarized in [Table A.1](#), dilutions were performed on all TNTC plates.

Despite performing 10-fold and 100-fold dilutions using Phosphate-Buffered Saline (PBS), samples continued to exhibit growth levels that exceeded countable limits. Photographs depicting two samples across the three plates—original 1 mL plate, 10-fold, and 100-fold dilutions—are presented in [Figure A.2](#) for visual reference.

3.2.2 Statistical Analysis of HPC Data

For the HPC analysis, we began by conducting the Mann-Whitney U test, also known as the Wilcoxon Rank Sum test. We used R for this analysis, and the code for performing this test is provided in [Appendix C](#). The test was applied to all 70 samples, which were split into two groups based on their respective flushing frequencies. This non-parametric test is suitable for data that does not follow a normal distribution and allows comparison between two groups without the assumption of any specific data distribution.

Hypotheses:

- Null Hypothesis (H_0): The distributions of bacterial counts in flushed and unflushed samples are identical.
- Alternative Hypothesis (H_1): The distribution of bacterial counts in flushed samples are not identical to the distribution of those from unflushed samples.

The resulting p-value was 0.1071. Since this p-value is greater than the commonly used significance level of $\alpha = 0.05$, this suggests that there is no statistically significant difference between the bacterial counts of the two groups at the 95% confidence interval.

To minimize the potential confounding effects of various variables in our study, we focused our second analysis on a subset of the data. We exclusively chose buildings where samples had been collected from both regularly flushed (RF) and irregularly flushed (IF) eyewash stations. This selective criterion was important to minimize variability influenced by factors such as building age, plumbing differences, and water source variations, which could otherwise alter the comparison between the two flushing routines. Due to the random nature of our sample collection, and the challenge of ensuring that both flushing types were sampled from

the same building, several buildings were excluded from our analysis. Ultimately, this left us with only 8 buildings for the analysis out of the initial pool of 21.

Using the same hypotheses on this selective set of samples, we performed the Wilcoxon rank sum test again. This time, the test revealed a statistically significant difference between the HPC levels in regularly flushed versus irregularly flushed eyewash stations at $\alpha = 0.01$, with a p-value of 0.009. These results are illustrated in Table 3.2 below.

Table 3.2 One-Tailed Wilcoxon Rank Sum Test for HPC Results

Comparison	Bacterial Counts	p-value	Observation
All Dataset (70 samples)	-	0.1	Difference is not statistically significant at the 95% Confidence Interval
Filtered Dataset*	Lower in regularly flushed	0.009	Difference is statistically significant

3.3 Pathogen Detection and Analysis

3.3.1 Legionella Detection and Enumeration

Out of the 70 samples tested, 4 samples from the same building yielded positive results for the presence of *Legionella pneumophila*. The details of the positive samples are presented below where the term “MPN” stands for Most Probable Number:

Table 3.3 Positive Legionella Detection Results

Legionella Result	MPN Count	HPC Result	Observations
Positive	2272.6	TNTC	General laboratory safety guidelines not followed
Positive	977.2	TNTC	General laboratory safety guidelines not followed
Positive	5.2	TNTC	Eyewash needed sink to be turned on, water bubbled out
Positive	180.1	TNTC	No Observations

The positive samples were all collected from the same building and came from irregularly flushed stations that tested as TNTC on the HPC test. The eyewash stations were functional in the first two labs, but field observations revealed non-compliance with UW’s

general laboratory safety standards. The eyewash station in the third lab shown in Table 3.3 above failed to operate properly, which after troubleshooting, was found to have water bubbling out from the eyewash when the sink's faucet was turned on. This malfunction indicates that the eyewash does not comply with occupational safety standards or plumbing codes that mandate independent and reliable operation of emergency eyewash stations.

3.3.2 Cultivation of Non-Tuberculosis Mycobacteria

3.3.2.1 Culturing Results for Non-Tuberculosis Mycobacteria

The data revealed considerable variability in colony types among the 37 samples cultured from eyewash stations, reflecting differences in the microbial communities across the sampled environments. All but one of the plates exhibited growth by week 8. The exception was a single eyewash station where the plate remained completely clean and white, suggesting either an absence of Mycobacterium or effective microbial control at that specific location. This clear white plate can be seen as the top far right plate in [Figure A.5](#). The presence of multiple colony colors within individual samples points to a complex biofilm, known for its ability to adhere to moist surfaces and to form heterogeneous communities.

3.3.2.2 Morphological Characteristics of Cultured Colonies

In the study of colonies cultured from eyewash stations, diverse morphological characteristics were observed and are summarized in [Table A.8](#) in the appendix. These colonies exhibited a range of growth patterns and textures, from rapid, expansive growth seen in red, yellow, and orange colonies, to isolated growth patterns seen in the clear and brown colonies. The textures of these colonies varied substantially, with some displaying a slimy consistency, particularly in red colonies, while others such as the white, yellow, beige, and brown colonies presented a drier texture. These qualitative assessments of colony texture were made during the removal of colonies for DNA extraction using an inoculation loop.

During the eight-week incubation period, a variety of growth patterns emerged across the cultured plates. Initially, one week after incubation, a rapid and expansive growth of red colonies was noted on multiple plates. The red colonies demonstrated a particularly vigorous and slimy growth, densely packed on some plates, while more distributed on others, despite their fast expansion. These red colonies maintained a moist texture, indicative of a potentially mucoid

biofilm formation. Additionally, during the first week, some plates unexpectedly supported the growth of mold. Yet, a few plates remained clear, showing no signs of microbial growth at that stage. By the second week, many of these plates were almost entirely covered with merged red colonies. The transformation continued into the third week, where plates that initially harbored white to beige colonies witnessed a progression to dark yellow colonies, spreading extensively across the surfaces. Notably, by the end of the second week, some plates initially dominated by red colonies began to exhibit new growth of white and yellow colonies, suggesting a sequential colonization where different microbial species or strains might interact or compete within the same habitat.

Following the eight-week incubation period, these colonies underwent DNA extraction and qPCR analysis to determine whether they were positive for *Mycobacterium*, with detailed results discussed in the following section.

3.4 Quantitative Polymerase Chain Reaction (qPCR) Analysis

3.4.1 Results of DNA Extraction from Plates for qPCR Analysis

[Table A.7](#) summarizes the qPCR analysis results from DNA extracted from various colonies cultured from eyewash stations. It details the outcome for each colony, whether it tested positive or negative for the presence of *Mycobacterium* and includes notations on both the colony color and the results of each colony type tested. The term “presumptive negative” refers to visually present colonies supposed to potentially contain *Mycobacterium* due to their growth and morphology but were ultimately not confirmed by qPCR. This term is used for samples that exhibit growth patterns suggestive of *Mycobacterium* but lack the specific genetic markers identified through qPCR. In this study, a plate is considered “positive” if at least one colony tests positive for *Mycobacterium*. Despite the application of cetylpyridinium chloride (CPC) to suppress non-target microbial growth, 37 out of 38 samples grew colonies on the plate after 8-week incubation period.

Table 3.4 *qPCR Analysis Results for Non-tuberculosis Mycobacterium*

Test Result	Regularly Flushed Stations	Irregularly Flushed Stations	Total
Positive for NTM	21	8	29
Negative for NTM	1	8	9
Total	22	16	38

3.4.2 Comparison of Mycobacteria Prevalence Across Different Facilities

Of the 38 cultured samples analyzed, only one plate remained clear after the 8-week incubation period, while the other 37 exhibited growth of one to four different colony types. Subsequent qPCR analysis of these samples revealed that 29 tested positives for Mycobacterium, whereas 8 tested negatives. The identification of specific strains such as *Mycobacterium chelonae* and *Mycobacterium avium complex* (MAC) from positive qPCR samples would require further genetic sequencing, which was not done in this study. Eight out of nine negative samples came from irregularly flushed eyewash stations, which also showed a ‘too numerous to count’ result on the heterotrophic plate count (HPC) test.

We conducted a Fisher’s Exact Test on these results with the following hypotheses:

- Null Hypothesis (H0): Regularly flushed eyewash stations do not have a higher likelihood of testing positive for mycobacteria compared to irregularly flushed stations.
- Alternative Hypothesis (H1): Regularly flushed eyewash stations have a higher likelihood of testing positive for mycobacteria compared to irregularly flushed stations.

With a p-value of 0.02 and a confidence interval: [1.03, 462.35], so it was statistically significant at the 95% confidence interval, the regularly flushed eyewash stations were more likely to test positive for mycobacterium. This will be elaborated on in the discussion section. For detailed R code used in performing these tests, please refer to [Appendix C](#).

3.5 External Lab Analysis: Amoeba Detection & Quantification

For the detection and quantification of *Acanthamoeba*, a portion of our samples was sent to an external laboratory. This laboratory is managed by Professor Tristan Jordan, an Assistant Professor in Microbiology at the University of Washington. Professor Jordan's lab is relatively new and was in the initial process of its operations during our collaboration. Due to these circumstances, the laboratory could only process four of our initial samples. None of these samples tested positive for *Acanthamoeba*. Due to delays at the external lab and the constrained timeline of our project, we were unable to proceed with the processing of additional samples.

Professor Jordan's laboratory processed our four initial samples, no *Acanthamoeba* was detected in any of the four samples. The qPCR analysis, tailored for high sensitivity, also failed to identify any *Acanthamoeba* DNA, confirming the absence of this pathogen in the tested samples. Also, the four samples tested negative for *Legionella pneumophila*, indicating no presence of this bacterium, which is known to be hosted by *Acanthamoeba*. The HPC for these samples also fell within acceptable limits, all registering below 500 colony-forming units per milliliter (CFU/mL), aligning with CDC's guidelines for water quality (CDC, 2015). These findings, although limited to a small subset of our total samples, provide useful information into the detection capabilities and challenges associated with *Acanthamoeba* identification in environmental samples.

Chapter IV: Discussion

4.1 Prevalence and Distribution

The results presented for HPC suggest that regular flushing of eyewash stations may reduce bacterial count. The HPC method was used to assess the presence of heterotrophic bacteria in the water samples collected. While HPC does not directly identify harmful bacteria, it serves as an indicator of potential contamination and reflects the overall microbial count, thus aiding in evaluating water quality (Sanchis et al., 2023). The Wilcoxon rank sum test performed on the buildings where we sampled both types of flushing routines from the same building revealed a statistically significant difference between the HPC levels in regularly flushed versus irregularly flushed eyewash stations at $\alpha = 0.01$, with a p-value of 0.009. This finding corroborates the hypothesis that regular flushing is effective in reducing microbial contamination. This analysis underscores the importance of regular maintenance routines in sustaining water quality and safety in emergency eyewash stations. With 99% confidence, our findings support the established best practices for emergency response equipment management and validate the necessity of proactive maintenance to minimize potential health risks associated with microbial contamination.

Regarding *Legionella* results, the test we conducted estimates the concentration of *Legionella* bacteria in water samples. Samples with MPNs of 2272.6 and 977.2 both show high levels of contamination, possibly posing a risk of exposure and necessitating immediate investigation and intervention to mitigate public health risks. Samples with lower MPNs of 5.2 and 180, suggest the presence of *Legionella* but at a lower concentration. The four positive samples for *Legionella pneumophila* were from the same building, specifically irregularly flushed stations that tested TNTC for the HPC test. The highest detected level of contamination reached an MPN value of 2272.6. This isolated occurrence provides a critical case study for understanding the conditions that favor the proliferation of *Legionella* in eyewash stations. This suggests the necessity for regular and thorough flushing of emergency eyewash stations as part of standard safety protocols.

In the NTM results, 8 out of 9 negative samples for Mycobacterium came from irregularly flushed stations. This observation suggests that natural microbial diversity and

competition within the pipes may influence which organisms thrive. Environmental factors or microbial interactions within the biofilm might inhibit the growth of Mycobacterium or possibly interfere with qPCR detection resulting in false negatives. The statistical analysis using a 2x2 table and a one-tailed Fisher's Exact Test verified that this counterintuitive trend that we observed was statistically significant (p -value = 0.02631). The confidence interval was extremely wide, though, which is due to the small number of samples and it indicates that this result is not precise and unreliable. In this pilot study, regularly flushed eyewash stations exhibited a higher likelihood of testing positive for mycobacteria compared to irregularly flushed stations. The findings of NTM challenge our hypothesis, but definitive conclusions cannot be drawn. Further research is needed, and recommended, to verify or refute these results.

4.2 Observations of Eyewash Stations and Water Quality in Sampled Stations

During the sampling phase, it was observed that compliance with eyewash safety standards varied across lab spaces. According to the log sheets provided by lab managers, building occupants, or building managers, some laboratories adhered to the prescribed safety protocols. However, it remains uncertain whether the flushing was performed correctly or sufficiently, as the exact duration of flushing was not verified. The actual conditions of some eyewash stations suggested discrepancies in routine safety practices. The overall cleanliness of labs appeared to depend significantly on their frequency of use, with less active labs showing signs of neglect. It became evident that there is a general lack of awareness regarding the importance of maintaining eyewash stations. Many lab personnel seem to underestimate the risks associated with contaminated eyewash stations. This oversight stresses the need for enhanced educational efforts to highlight the role of eyewashes in lab safety and to ensure they are kept in acceptable condition.

Observations of the water quality in sampled eyewash stations revealed various issues. The color of the water collected varied, with some samples showing an orange to yellowish shade, which is possibly indicating rust or chemical residues. Several bottles contained visible sediments settling at the bottom, with rust and what appeared to be salt, ranging in color from black and orange to red and rust-like tones. This diversity in sediment coloration could point to various types of corrosion and mineral accumulation, each potentially harmful and indicative of various water quality issues. Also, small black debris was noted in other samples, which could be

attributed to a variety of sources including particles from the air or disintegration of internal components of the pipes or eyewash fixtures, occurring if maintenance is neglected. Such findings highlight the critical need for regular and thorough maintenance of eyewash stations to ensure they remain safe in emergency situations.

4.3 Key Findings and Implications

4.3.1 Implications of Findings for Workplace Safety and Public Health

The findings of this study underscore the critical role of maintenance in ensuring the effectiveness of emergency eyewash stations in workplace environments. Regular flushing of eyewash stations not only maintains water quality but also reduces the risk of potential eye infections that could result from exposure to contaminated water. This is particularly important in settings where workers are routinely exposed to hazardous chemicals or materials. The presence of pathogens such as *Legionella pneumophila* and non-tuberculous Mycobacterium in some eyewash stations indicates a potential health risk, not only to the eyes but also to the respiratory systems if aerosolized particles are inhaled (CDC,2024; OSHA, 2015; Swanson et al., 2023).

The detection of high levels of *Legionella* in systems with irregular flushing schedules highlights the importance of adhering to ANSI Z358.1-2014 standards, which recommend weekly activations to prevent *Legionella* proliferation (OSHA, 2015). Given these insights, facility managers should enforce ANSI standards more rigorously. This is not about bureaucratic compliance, but about protecting public health and ensuring the safety of individuals in environments with the potential for chemical exposures.

4.3.2 Effectiveness of Flushing Routines

The persistence of high counts for HPC and pathogens like non-tuberculosis Mycobacterium in regularly flushed stations suggests that while flushing is necessary, it may not be sufficient on its own. TNTC results in the regularly flushed stations were more than expected. This observation suggests that flushing alone is not the only factor influencing bacterial levels. The potential reasons for these inconsistencies could be due to improper practices such as not flushing the eyewashes for the required amount of time, reporting biases, or other factors such as the water system and pipes. For the Mycobacterium samples there could be natural variability or

unique ecological conditions within certain pipes that might affect results. Other confounding factors might be variability stemming from the age of buildings or differences in water sources, which may be impacting our results as well. Despite these complexities, the Wilcoxon test did indicate statistical significance for the select buildings at the 99% confidence level, that regular flushing eyewash station significantly lowers microbial levels.

4.3.3 Discussion and Integration with Existing Literature

The findings of this study align with existing literature on occupational health and safety. Loret and Dumoutier (2019) noted the importance of regular maintenance and flushing of eyewash stations to prevent microbial contamination. Research, such as that by Swanson et al. (2023), also details the risks associated with nontuberculous Mycobacterium populations in eyewash stations, particularly in units with prolonged water stagnation. These pathogens can lead to health risks, as mentioned by OSHA, when workers with existing eye injuries or compromised immune systems use these facilities (Swanson et al., 2023).

Legionella, known for causing serious pulmonary diseases, can thrive in stagnant water conditions typical of poorly maintained eyewash stations. While *Legionella* does not directly cause eye infections, the inhalation of aerosols containing these bacteria from contaminated eyewash stations can lead to Legionnaires' disease, a severe and potentially fatal condition. So, monitoring and testing should be part of the regular maintenance routines of eyewash stations, as evidenced by the work of Paszko et al. (1998), which advocates for comprehensive management strategies that go beyond simple flushing (Paszko-Kolva et al., 1998).

Studies such as those by Thomson et al. (2007) have documented that NTM, while traditionally associated with pulmonary conditions, can also pose risks in any setting where water aerosols might be inhaled or ingested (Thomson et al., 2007). The consistent theme across these studies is the necessity of stringent adherence to safety standards recommended by organizations like ANSI and OSHA, which aim to safeguard the health of individuals potentially exposed to hazardous conditions by inadequate emergency response facilities.

4.4 Limitations

While providing valuable insights into the maintenance of eyewash stations, this study, had several limitations that may affect the interpretation of the results. One primary constraint

was the small sample size and the limited number of buildings from which samples from both flushing routines were collected. This limited the ability to conduct a comparative analysis between regularly and irregularly flushed eyewash stations.

A notable methodological limitation was the identification of nontuberculous mycobacteria (NTM) types present in the eyewash stations. Although the qPCR analysis effectively confirmed the presence of NTM, the study did not extend to genetic sequencing, which would have allowed for the differentiation between specific strains, such as *Mycobacterium chelonae* or *Mycobacterium avium complex* (MAC). Also, the microbial detection methods employed were limited to heterotrophic plate counts, *Legionella*, and NTM. Consequently, this approach failed to detect a broader spectrum of potentially harmful pathogens, overlooking other organisms that could pose significant health concerns but were not targeted or detected due to the scope of the methods used.

Moreover, the reliance on self-reported data for flushing frequencies and methods introduces another layer of uncertainty. There is an important risk of reporting bias or not adhering to the correct flushing techniques or the recommended duration. Such shortcomings can lead to data that does not accurately reflect true maintenance practices, thus skewing the study's outcomes. Which is why this study should be complemented with further research using more samples, more diverse building types, and advanced microbial detection techniques.

4.5 Conclusions & Recommendations

4.5.1 Future Research Directions

Further research will enable a broader statistical analysis and allow for more accurate comparisons between and within buildings with both regularly and irregularly flushed eyewash stations. It is important to ensure an equal number of samples are collected from each building, and that samples are gathered equally from both regularly and irregularly flushed eyewash stations to account for building-specific variables.

It is crucial to implement longitudinal monitoring of maintenance routines to observe changes in microbial contamination levels over time. For instance, collecting samples before and after flushing—at immediate, 5 minutes later, and 10 minutes later intervals—will help determine the optimal flushing duration needed to effectively reduce microbial levels.

Engaging with laboratory occupants and maintenance personnel is also essential to assess their awareness and adherence to recommended flushing durations. This assessment will help identify any discrepancies between reported and actual practices. Moreover, experimenting with different flushing regimes, such as maintaining eyewash stations with regular flushes over a month or two and then re-testing, will provide insights into the long-term effectiveness of such flush.

4.5.2 Recommendations for Practice

To improve the safety and functionality of emergency eyewash stations within the workplace, the following comprehensive set of recommendations is proposed based on the findings of this study: First, it is crucial to conduct more frequent inspections, this is to make sure standards are properly enforced and meticulously followed. Such inspections can be randomized to make sure all eyewashes in the institution are tested occasionally and can be ramped up or ramped down depending on the results.

Testing the current eyewash stations regularly and cleaning them as needed will help maintain their functionality and safety. So, it is recommended to establish a maintenance routine that includes regular and mandatory flushing of the eyewash stations, especially given that some labs were irregularly flushing their eyewash stations. Ensuring that the eyewash stations are in good condition is also essential; this includes verifying that they do not have broken caps and that the caps are securely closed to prevent debris from entering the station.

Moreover, one recommendation is to enforce a longer and mandated flushing period. 10 minutes of flushing have been shown to be effective in reducing microbial contamination significantly (Swanson et al., 2023). More consideration can be made to decide on an appropriate flushing duration per institution or per building, up to 10 minutes, based on their needs. Additionally, ensuring that eyewash stations are hands-free upon operation is crucial in emergency situations to guarantee immediate and unrestricted access for users. Educating workers about the importance of eyewash station maintenance and the potential health risks associated with contaminated stations is fundamental. This education can enhance workers' understanding and adherence to safety protocols, reducing the risk of misuse and neglect.

Finally, at least compliance with ANSI/OSHA standards must be strictly enforced, and consideration should be given to adopting more stringent standards where frequent issues with

eyewash station safety and effectiveness are detected. By addressing these recommendations, workplaces can significantly enhance the safety of their emergency eyewash stations, thereby reducing the risk of contamination and improving overall workplace safety. Implementing these practices will not only comply with regulatory requirements but also promote a safer and more responsive working environment.

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Appendices
Appendix A: Additional Tables and Figures

Table A.1 – Field Data Collected & HPC Results

Sample ID	Date Collected	Flushing Routine	Date of Analysis	HPC CFU/ml
L1	09/12/2023	Irregularly Flushed	09/14/2023	71
L2	09/12/2023	Irregularly Flushed	09/14/2023	28
L3	09/12/2023	Irregularly Flushed	09/14/2023	130
L4	09/12/2023	Irregularly Flushed	09/14/2023	TNTC
L5	09/12/2023	Irregularly Flushed	09/14/2023	86
L6	09/12/2023	Irregularly Flushed	09/14/2023	TNTC
L7	09/12/2023	Irregularly Flushed	09/14/2023	TNTC
L8	09/12/2023	Regularly Flushed	09/14/2023	54
L9	09/19/2023	Regularly Flushed	09/21/2023	9
L10	09/19/2023	Regularly Flushed	09/21/2023	66
L11	09/19/2023	Regularly Flushed	09/21/2023	3
L12	10/12/2023	Regularly Flushed	10/12/2023	1
L13	09/20/2023	Irregularly Flushed	09/21/2023	TNTC
L14	09/26/2023	Irregularly Flushed	09/28/2023	TNTC
L15	09/26/2023	Irregularly Flushed	09/28/2023	6
L16	09/28/2023	Irregularly Flushed	09/28/2023	0
L17	10/03/2023	Regularly Flushed	10/05/2023	0
L18	10/03/2023	Regularly Flushed	10/05/2023	34
L19	10/03/2023	Regularly Flushed	10/05/2023	TNTC
L20	10/10/2023	Regularly Flushed	10/12/2023	TNTC
L21	10/10/2023	Regularly Flushed	10/12/2023	TNTC
L22	10/11/2023	Regularly Flushed	10/12/2023	TNTC
L23	10/11/2023	Regularly Flushed	10/12/2023	TNTC
L24	10/11/2023	Regularly Flushed	10/12/2023	4
L25	10/11/2023	Regularly Flushed	10/12/2023	TNTC
L26	10/11/2023	Regularly Flushed	10/12/2023	TNTC
L27	10/17/2023	Irregularly Flushed	10/19/2023	TNTC
L28	10/17/2023	Irregularly Flushed	10/19/2023	TNTC
L29	10/17/2023	Irregularly Flushed	10/19/2023	TNTC
L30	10/17/2023	Regularly Flushed	10/19/2023	29
L31	10/17/2023	Regularly Flushed	10/19/2023	1
L32	01/22/2024	Regularly Flushed	01/24/2024	110
L33	01/22/2024	Regularly Flushed	01/24/2024	77
L34	01/22/2024	Irregularly Flushed	01/24/2024	TNTC
L35	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L36	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L37	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L38	01/22/2024	Irregularly Flushed	01/24/2024	TNTC

L39	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L40	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L41	01/22/2024	Irregularly Flushed	01/24/2024	TNTC
L42	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L43	01/22/2024	Irregularly Flushed	01/24/2024	TNTC
L44	01/22/2024	Regularly Flushed	01/24/2024	TNTC
L45	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L46	01/29/2024	Regularly Flushed	01/31/2024	4
L47	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L48	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L49	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L50	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L51	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L52	01/29/2024	Regularly Flushed	01/31/2024	160
L53	01/29/2024	Regularly Flushed	01/31/2024	TNTC
L54	01/29/2024	Irregularly Flushed	01/31/2024	TNTC
L55	01/29/2024	Regularly Flushed	01/31/2024	87
L56	01/29/2024	Irregularly Flushed	01/31/2024	TNTC
L57	01/31/2024	Regularly Flushed	01/31/2024	TNTC
L58	01/31/2024	Irregularly Flushed	01/31/2024	TNTC
L59	01/31/2024	Irregularly Flushed	01/31/2024	TNTC
L60	01/31/2024	Regularly Flushed	01/31/2024	15
H1	09/19/2023	Irregularly Flushed	09/21/2023	0
H2	09/19/2023	Irregularly Flushed	09/21/2023	TNTC
H3	09/19/2023	Irregularly Flushed	09/21/2023	TNTC
H4	09/19/2023	Irregularly Flushed	09/21/2023	TNTC
H5	10/03/2023	Irregularly Flushed	10/05/2023	TNTC
H6	10/03/2023	Irregularly Flushed	10/05/2023	TNTC
H7	10/03/2023	Irregularly Flushed	10/05/2023	TNTC
H8	01/29/2024	Regularly Flushed	01/31/2024	TNTC
H9	01/29/2024	Regularly Flushed	01/31/2024	TNTC
H10	01/29/2024	Regularly Flushed	01/31/2024	TNTC

Figure A.2 – Variations in Water Quality Across Four Eyewash Station Samples



Photo Credit: Mariam Assaad

Figure A.3 – Visual Representation of Bacterial Growth on Original, 10-Fold, and 100-Fold Dilution Plates for Two TNTC Samples

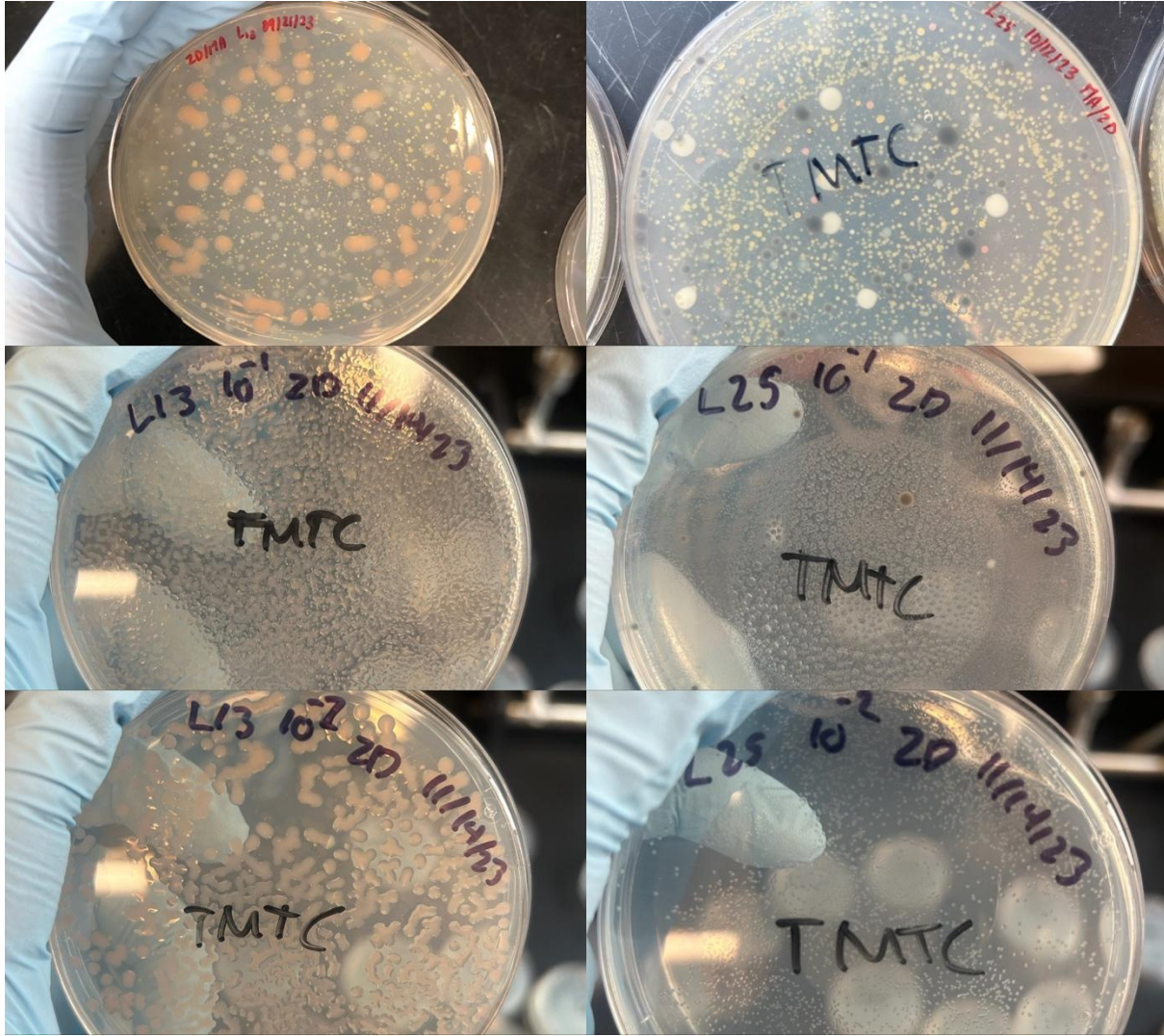


Photo Credit: Mariam Assaad

Table A.4 – Culturing Observations of Non-Tuberculosis Mycobacteria

Sample ID	Type of Colonies Observed
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L1	2 (Red, White)
L2	2 (Red, White)
L3	3 (Red, White, Yellow)
L4	2 (Red, White)
L5	3 (Red, White, Yellow)
L6	2 (Red, Beige)
L7	2 (Red, Beige)
L8	3 (Red, Beige, Yellow)
L9	4 (White, Beige, Yellow, Orange)
L10	2 (Beige, White)
L11	1 (Yellow)
L12	1 (Yellow)
L13	2 (Red, Purple)
L14	2 (Beige, Brown)
L15	4 (Red, Beige, Yellow, Orange)
L16	2 (Beige, Orange)
L17	3 (Red, Beige, Yellow)
L18	3 (Red, Beige, Orange)
L19	4 (Red, Beige, Yellow, Orange)
L20	3 (Red, Yellow, Orange)
L21	3 (Brown, White, Orange)
L22	3 (Red, White, Orange)
L23	4 (Red, Beige, Yellow, White)
L24	4 (White, Beige, Yellow, Orange)
L25	3 (White, Beige, Orange)
L26	4 (White, Clear, Red, Orange)
L27	2 (Beige, Yellow)
L28	1 (Brown)
L29	1 (Beige)
L30	Positive
H1	2 (Yellow, Red)
H2	1 (Red)
H3	1 (Red)
H4	1 (Red, Beige)
H5	1 (Red)
H6	3 (Orange, Red, Beige)
H7	1 (Beige)

Figure A.5 – *Growth of Non-Tuberculosis Mycobacteria on Culture Plates After 8 Weeks of Incubation*



Photo Credit: Mariam Assaad

Figure A.6 Colony Morphologies on Two Different Plates

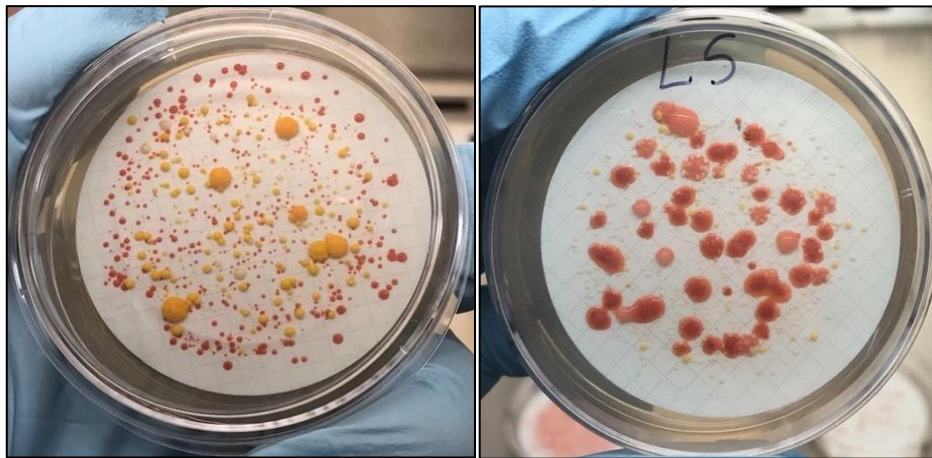


Photo Credit: Mariam Assaad

Table A.7 – qPCR Analysis of Extracted Cultured Colonies

Sample ID	qPCR Result	Notes
L1	Positive	Positive in red colony, negative in white colony
L2	Positive	Positive in red and white colonies
L3	Positive	Positive in orange, red, and white colonies
L4	Positive	Positive in white colony, negative in red colony
L5	Positive	Positive in red, white, and yellow colonies
L6	Positive	Positive in beige colony, presumptive negative in red colony
L7	NEGATIVE	Negative in red and beige colonies
L8	Positive	Positive in red, beige, and yellow colonies
L9	Positive	Positive in orange, beige, white, and yellow colonies
L10	Positive	Positive in beige colony, negative in white colony
L11	Positive	Positive in yellow colony
L12	Positive	Positive in yellow colony

L13	NEGATIVE	Negative in red and purple colonies
L14	NEGATIVE	Negative in brown and beige colonies
L15	Positive	Positive in orange and yellow colonies; negative in red and beige colonies
L16	Positive	Positive in orange and beige colonies
L17	Positive	Positive in yellow and beige colonies; presumptive negative in red colony
L18	Positive	Positive in orange, beige, and white colonies; negative in red colony
L19	Positive	Positive in orange, red, and yellow colonies; negative in beige colony
L20	Positive	Positive in orange, red, and yellow colonies
L21	Positive	Positive in orange, white, and brown colonies
L22	Positive	Positive in orange, red, and white colonies
L23	Positive	Positive in yellow colony; negative in beige, red, and white colonies
L24	Positive	Positive in orange, beige, white, and yellow colonies
L25	Positive	Positive in orange, beige, and white colonies
L26	Positive	Positive in orange and white colonies; negative in a clear color and red colonies
L27	Positive	Positive in beige and yellow colonies
L28	Positive	Positive in brown colony
L29	NEGATIVE	Negative in beige colony
L30	Positive	Positive in red colony
H1	Positive	Positive in yellow colony, negative in red colony
H2	NEGATIVE	Negative in red colony
H3	Positive	Negative in red colony
H4	NEGATIVE	Negative in red and beige colony
H5	NEGATIVE	Negative in red colony
H6	Positive	Positive in orange and red colonies, Presumptive negative in beige colony
H7	Presumptive NEGATIVE	Presumptive negative in beige colony

Table A.8 – Morphological Characteristics of Diverse Colonies Cultured from Eyewash Stations

Colony Type	Growth Pattern	Colony Texture
Red	Rapid, expansive	Some colonies merged together, and have a slimy consistency
Yellow	Rapid, expansive	Colonies are coarse
Orange	Rapid growth	Rough texture
White	Moderate growth	Smaller colonies near larger ones, with a dry texture
Clear	Isolated	Few colonies that are partially see-through
Beige	Moderate growth	Few colonies with a smooth surface
Brown	Isolated growth	Growth confined to a small area with a dry texture and dark brown color

Appendix B: Miscellaneous Data

B.1 – Sampling Outreach Document

Eyewash Water Sampling Outreach

Hello,

This message is a request on behalf of the UW School of Public Health to include your space as part of an environmental sampling study that we are conducting on campus. We plan to collect samples from your building on one of the following dates 01/22/2024, 01/29/2024, and 02/05/2024.

Who we are: Members from the Environmental and Occupational Health Lab (EOHML) in the UW School of Public Health's Department of Environmental and Occupational Health Sciences. Our lab investigates the presence, control, and exposure to microorganisms in environmental media including water, air, soil, and food.

What we are evaluating: We would like to understand the baseline water quality of emergency eyewashes across UW campus that are tested and therefore flushed regularly, and those that are flushed less frequently. This will help us understand how effective the current eyewash testing/flushing recommendations are. We will be evaluating the amount and types of microorganisms present in these eyewashes.

How we are sampling: Our research team will collect a water sample from up to three eyewashes in your building. A 1-liter water sample will be collected from your eyewash into a sterile bottle that we will provide. We ask that you DO NOT flush your eyewash prior to us taking the sample. If available, we would like copy of the eyewash testing log. Samples will be taken to the EOHML for analysis over the coming weeks. We anticipate that it might take up to 12 weeks to receive the results. Our team has already completed this process for several buildings during Fall Quarter.

What happens with our results: We will share any results that fall outside of expected ranges for microbial growth with you, your building coordinator, UW Facilities, and the UW Environmental Health & Safety Department (EH&S). We are coordinating with all of these parties throughout the project. If any findings are outside of expected ranges, EH&S will be made aware will contact you about next steps. Published results from our research will not be publicly linked to your lab in any way. A copy of your lab's full eyewash sampling results can be provided in any case, upon request.

What we need from you:

- To schedule 5 minutes with the Principal Investigator, Lab Manager (or designed staff member) to give us access to your eye wash(es) and answer question about their use. We ask to be escorted in your lab while we collect samples.
- We ask water collection will be scheduled during a time when research activities are not taking place.
- Information on any PPE we should wear in order to access your laboratory and the eyewash.
- Access to your eyewash flush record, if available.

Please let us know if you have any questions regarding this project and our activities in your building. We look forward to hopefully visiting your lab space.

Contact Info:

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B.2 – 96-Well Quanti-Tray/Legiolert MPN Table

96-Well Quanti-Tray/Legiolert[®] MPN Table

	# Large Wells Positive						
	0	1	2	3	4	5	6
0	0	1	2	3	4	5	6
1	0	2	3	5	7	10	15
2	2	3	7	8	9	12	15
3	3	4	5	7	10	14	22
4	4	5	7	9	13	15	25
5	5	6	8	10	14	19	30
6	6	7	9	12	16	21	30
7	7	8	10	13	17	24	31
8	8	9	12	15	18	25	31
9	9	11	13	16	19	26	32
10	10	12	15	18	20	27	33
11	11	13	16	19	21	28	34
12	12	14	17	20	22	29	35
13	13	15	18	21	23	30	36
14	14	16	19	22	24	31	37
15	15	17	20	23	25	32	38
16	16	18	21	24	26	33	39
17	17	19	22	25	27	34	40
18	18	20	23	26	28	35	41
19	19	21	24	27	29	36	42
20	20	22	25	28	30	37	43
21	21	23	26	29	31	38	44
22	22	24	27	30	32	39	45
23	23	25	28	31	33	40	46
24	24	26	29	32	34	41	47
25	25	27	30	33	35	42	48
26	26	28	31	34	36	43	49
27	27	29	32	35	37	44	50
28	28	30	33	36	38	45	51
29	29	31	34	37	39	46	52
30	30	32	35	38	40	47	53
31	31	33	36	39	41	48	54
32	32	34	37	40	42	49	55
33	33	35	38	41	43	50	56
34	34	36	39	42	44	51	57
35	35	37	40	43	45	52	58
36	36	38	41	44	46	53	59
37	37	39	42	45	47	54	60
38	38	40	43	46	48	55	61
39	39	41	44	47	49	56	62
40	40	42	45	48	50	57	63
41	41	43	46	49	51	58	64
42	42	44	47	50	52	59	65
43	43	45	48	51	53	60	66
44	44	46	49	52	54	61	67
45	45	47	50	53	55	62	68
46	46	48	51	54	56	63	69
47	47	49	52	55	57	64	70
48	48	50	53	56	58	65	71
49	49	51	54	57	59	66	72
50	50	52	55	58	60	67	73
51	51	53	56	59	61	68	74
52	52	54	57	60	62	69	75
53	53	55	58	61	63	70	76
54	54	56	59	62	64	71	77
55	55	57	60	63	65	72	78
56	56	58	61	64	66	73	79
57	57	59	62	65	67	74	80
58	58	60	63	66	68	75	81
59	59	61	64	67	69	76	82
60	60	62	65	68	70	77	83
61	61	63	66	69	71	78	84
62	62	64	67	70	72	79	85
63	63	65	68	71	73	80	86
64	64	66	69	72	74	81	87
65	65	67	70	73	75	82	88
66	66	68	71	74	76	83	89
67	67	69	72	75	77	84	90
68	68	70	73	76	78	85	91
69	69	71	74	77	79	86	92
70	70	72	75	78	80	87	93
71	71	73	76	79	81	88	94
72	72	74	77	80	82	89	95
73	73	75	78	81	83	90	96
74	74	76	79	82	84	91	97
75	75	77	80	83	85	92	98
76	76	78	81	84	86	93	99
77	77	79	82	85	87	94	100
78	78	80	83	86	88	95	101
79	79	81	84	87	89	96	102
80	80	82	85	88	90	97	103
81	81	83	86	89	91	98	104
82	82	84	87	90	92	99	105
83	83	85	88	91	93	100	106
84	84	86	89	92	94	101	107
85	85	87	90	93	95	102	108
86	86	88	91	94	96	103	109
87	87	89	92	95	97	104	110
88	88	90	93	96	98	105	111
89	89	91	94	97	99	106	112
90	90	92	95	98	100	107	113
91	91	93	96	99	101	108	114
92	92	94	97	100	102	109	115
93	93	95	98	101	103	110	116
94	94	96	99	102	104	111	117
95	95	97	100	103	105	112	118
96	96	98	101	104	106	113	119
97	97	99	102	105	107	114	120
98	98	100	103	106	108	115	121
99	99	101	104	107	109	116	122
100	100	102	105	108	110	117	123

B.3 – qPCR Spreadsheet

Date:	4/3/24							
Project:	Eyewash project							
Samples:								
Citation:	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4219376/#S2							
Objective:	Mycobacterium detection from colony extracts							
Reagents:								
	Final concn		Vol/Rxn		Vol/	96	Rxn	
iTaq universal probe supermix			10.0			1008.0		
atpE-Forward	10 uM		1.6			161.3		
atpE-Reverse	10 uM		1.6			161.3		
Probe (atpE)	1 uM		1.058			106.6		
H ₂ O			3.24			326.8		
DNA			2.50			252.0		
			20.0	µl/Rxn	Total:	1764.0	µl	
					Add:	17.5	µl	
Total RNA			2.50					

Appendix C: R Code

```
> # HPC Analysis R Code

library(readxl)
Thesis_R_Script <- read_excel("ThesisResults.xlsx")
library(tidyverse)
library(dplyr)

> regularly_flushed_hpc <- Thesis_R_Script %>%
+   filter(flushing_protocol == "RF") %>%
+   mutate(HPC = as.numeric(replace(HPC, HPC == "TNTC", 500))) %>%
+   pull(HPC)
> irregularly_flushed_hpc <- Thesis_R_Script %>%
+   filter(flushing_protocol == "IR") %>%
+   mutate(HPC = as.numeric(replace(HPC, HPC == "TNTC", 500))) %>%
+   pull(HPC)
> test_result <- wilcox.test(regularly_flushed_hpc, irregularly_flushed_hpc,
alternative = "less", exact = FALSE)
> print(test_result)

      Wilcoxon rank sum test with continuity correction

data:  regularly_flushed_hpc and irregularly_flushed_hpc
W = 507, p-value = 0.1071
alternative hypothesis: true location shift is less than 0

> # Count occurrences using dplyr, grouped by Building and Flushing Protocol
> buildings_RF_IRF_count <- Thesis_R_Script %>%
+   group_by(Building, Flushing_Protocol) %>%
+   summarise(Count = n())

> # Filter out buildings that appear only once because they include either
only regularly flushed or only irregularly flushed eyewashes
> filtered_buildings <- buildings_RF_IRF_count %>%
+   group_by(Building) %>%
+   mutate(Count2 = n()) %>%
+   filter(Count2 > 1) %>%
+   select(-Count2)

> # Print the filtered data
> print(filtered_buildings)
# A tibble: 16 × 3
# Groups:   Building [8]
  Building Flushing_Protocol Count
<chr>      <chr>          <int>
1 B1        IR                1
2 B1        RF                2
3 B2        IR                3
4 B2        RF                1
5 B3        IR                3
6 B3        RF                3
7 B4        IR                1
8 B4        RF                2
9 B5        IR                2
10 B5       RF                2
```

```

11 B6          IR          2
12 B6          RF          2
13 B7          IR          3
14 B7          RF          6
15 B8          IR          2
16 B8          RF          1

```

```

> # Filter the original dataframe to include only the buildings in the above
list

```

```

> filtered_results <- Thesis_R_Script %>%
+   filter(Building %in% filtered_buildings$Building)

```

```

> # Filter the dataframe to include only "Regularly Flushed" samples
> # Convert 'TNTC' to 500, then extract the HPC column as a numeric vector
> regularly_flushed_hpc <- filtered_results %>%
+   filter(Flushing_Protocol == "RF") %>%
+   mutate(HPC = as.numeric(replace(HPC, HPC == "TNTC", 500))) %>%
+   pull(HPC)

```

```

> # Print the resulting vector of HPC values

```

```

> print(regularly_flushed_hpc)
[1]  9 66  3  1 500 29  1 110 77 500 500 500 500 500 500 500 87 500
15

```

```

> # Filter the dataframe to include only "Irregularly Flushed" samples
> # Convert 'TNTC' to 500, then extract the HPC column as a numeric vector
> irregularly_flushed_hpc <- filtered_results %>%
+   filter(Flushing_Protocol == "IR") %>%
+   mutate(HPC = as.numeric(replace(HPC, HPC == "TNTC", 500))) %>%
+   pull(HPC)

```

```

> # Print the resulting vector of HPC values

```

```

> print(irregularly_flushed_hpc)
[1] 71 500  0 500 500 500 500 500 500 500 500 500 500 500 500 500

```

```

> # Perform the Mann-Whitney U test (one-sided: less)
> # Group RF is believed to have a lower median than Group IRF
> test_result <- wilcox.test(regularly_flushed_hpc, irregularly_flushed_hpc,
alternative = "less", exact = FALSE)

```

```

> # Print the results
> print(test_result)

```

Wilcoxon rank sum test with continuity correction

```

data: regularly_flushed_hpc and irregularly_flushed_hpc
W = 98.5, p-value = 0.009122
alternative hypothesis is true

```

```

> # Mycobacterium Analysis R Code

```

```

> # Data frame is named Thesis_R_Script
> # Columns are named Flushing_Protocol and Mycobacteria
>

```

```

> # Create the contingency table
> mycobacteria_table <- table(Thesis_R_Script$Flushing_Protocol,
Thesis_R_Script$Mycobacteria)

> # Print the table
> print(mycobacteria_table)

      Negative Positive Presumptive Negative
IR          7         13                1
RF          1         16                0

> # Modify the mycobacteria results to combine "Presumptive Negative" with
"Negative"
> Thesis_R_Script <- Thesis_R_Script %>%
+   mutate(Mycobacteria = ifelse(Mycobacteria == "Presumptive Negative",
"Negative", Mycobacteria))
> mycobacteria_table <- table(Thesis_R_Script$Flushing_Protocol,
Thesis_R_Script$Mycobacteria)

> print(mycobacteria_table)

      Negative Positive
IR          8         13
RF          1         16

> fisher_test <- fisher.test(mycobacteria_table)
> print(fisher_test)

```

Fisher's Exact Test for Count Data

```

data: mycobacteria_table
p-value = 0.02631
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 1.027069 462.351786
sample estimates:
odds ratio
 9.332426

```