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Isolation of Legionella Pneumophila From Well-Maintained Emergency Showers and Eyewash Stations

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ISOLATION OF *LEGIONELLA PNEUMOPHILA* FROM WELL-MAINTAINED EMERGENCY SHOWER AND EYEWASH STATIONS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

By

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Abstract

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Legionella pneumophila is a gram-negative bacterium responsible for Legionnaire's disease, and is commonly transmitted via aerosolized water. *Legionella* colonization of emergency eyewash and shower stations may pose an exposure hazard to users of these stations. There is little information about the role of these stations as significant reservoirs for *Legionella*. Samples were collected from 67 stations in an industrial facility. At the time of this study, the stations within this facility were under a routine maintenance program that included at least monthly flushing. This study also included the analysis for other bacterial organisms to determine an association between the presence and concentration of other bacteria and *Legionella*. All samples resulted in no detection of *Legionella,* yet 12 of the samples contained large counts of other bacteria. Thus, this study supports that properly maintained emergency eyewash and shower stations do not appear to be a significant source for aerosol transmission of *Legionella.*

Chapter 1 Introduction

Legionella pneumophila is a gram-negative, non-spore-forming aerobic bacterial organism. Found naturally in the environment, this organism is widely distributed in aquatic habitats such as surface waters in lakes, streams, and rivers.[8,14,17] It grows best in warm water with an approximate temperature range of 20°C to 45°C.[8] However, these organisms may remain dormant in cool water and proliferate when conditions become more favorable.[8]

L. pneumophila has at least 15 sero groups. This study focused on the *L. pneumophila* sero group 1, the one most responsible for human disease.[5,13] The most common mode of transmission to humans is exposure to aerosolized water or water mists from contaminated hot tubs, cooling towers, hot water tanks, air conditioning systems, humidifiers, showers, wash stands, and sinks.[5,6,8] These pieces of equipment may have also received contaminated water from the large plumbing systems that supply water to them.

Within water plumbing systems, main sites of colonization and concentrations of *Legionella* are highest in biofilms, located along the lining of piping, at fittings, and openings of water outlets.[5] Biofilm is made up of a collection of microorganisms, which create a layer of slime on surfaces in constant contact with water. These microorganisms are primarily bacteria that feed on scale and protozoa that feed on the bacteria. The

Occupational Safety and Health Administration (OSHA) describes the association between the biofilm and *Legionella* bacteria as a nourishing and sheltering resource.[17] The other bacterial organisms within the biofilm act as a nutrient source while the protozoa organisms serve as a host by harboring the *Legionella* bacteria. *Legionella* is considered an intracellular pathogen and, when consumed by a protozoan, is able to avoid phagocytosis within the protozoan host cell, allowing for intracellular replication. The infected host cell then undergoes apoptosis, and the newly replicated bacteria get released back into the environment. Potential protozoan hosts include a few species of ciliated protozoa and several species of amoebae.[11]

Water plumbing systems with stagnant areas (dead zones) promote biofilm accumulation and conditions conducive to bacterial colonization. Areas with significant biofilm can promote higher levels of *Legionella*.[8] There exists no exact level that constitutes what a "significant" biofilm level may be; however a review by the World Health Organization suggests that concentrations of other bacteria greater than 100 colony-forming units per milliliter (cfu/ml) of water may accompany the appearance of *Legionella*.[5] Shelton et al. suggest that as few as 10 cfu/ml of *Legionella* in potable water constitutes an uncommonly high level of contamination.[19]

Upon use of a contaminated system, the flow of water may dislodge biofilm organisms and carry them through the outlet where they remain with the water droplets and become aerosolized. Individuals most susceptible to *L. pneumophila* are those with compromised or suppressed immune systems, notably hospital patients exposed to contaminated hot-water plumbing systems throughout the facility, as well as individuals

with compromised respiratory systems such as smokers.[5,6,8] Any system or water reservoir that has the potential to aerosolize water could possibly release *Legionella* if the agent is contained within the water source.[19]

Legionella are exceptional bacteria with a higher tolerance for chlorine than most other bacteria.[5] This tolerance is further enhanced when the bacterium is within the protective shelter of a host protozoan. Therefore, residual chlorine carried through the public water supply may not prevent growth of the organism.[5] The American National Standards Institute recommends that water systems, such as emergency eyewash and shower stations, be activated weekly for a period long enough to clear the supply line and minimize microbial contamination.[1] Poorly maintained systems are more likely to offer favorable conditions for colonization with *Legionella* than more well-maintained systems.

Large water plumbing systems are more likely to become colonized by *Legionella* due to the potential of a larger biofilm bearing surface available for bacterial growth.[5] Because of the long network of piping, stationary emergency showers and eyewash stations may serve as potential reservoirs for *Legionella* if they remain unused for prolonged periods. Lack of use or water flow throughout water plumbing systems allows for stagnation and a great opportunity for bacterial amplification. However, because water in these systems is cold or ambient in temperature, *L. pneumophila* may stay dormant and may not be able to proliferate into detectable numbers. This may be why there has been little implication that these stations serve as reservoirs or are a cause for disease.[17] Yet, a study conducted in 1990 on 40 eyewash stations found detectable levels of *Legionella* in 35 of the stations.[18] This study concluded that when not regularly flushed

and/or cleaned, eyewash stations may be a source of bacterial contamination. The proposed hypothesis to test is whether emergency eyewash stations and combination eyewash and shower stations, under a strict maintenance regimen, serve as a significant source of *Legionella,* and whether they pose a health risk from exposure to users.

Chapter 2 Approach and Methods

Research was conducted at an industrial facility containing 257 eyewash stations and combination eyewash and shower stations. Sampling was conducted for both *Legionella* and other bacterial organisms within these stations. Water and swab samples were collected for the detection of *Legionella*, to include both the free form in water and the sheltered form within potential biofilm deposits. Also tested was potential accumulation of biofilm through the detection of other bacterial growth to understand whether a correlation between the levels of other bacteria and the detection of *Legionella* exists. Currently, there is no known evidence indicating that the growth of any particular bacterial species correlates with the presence of *Legionella.* As a result, variations of swab and water sampling were conducted for the detection of other types of bacteria.

The six buildings of interest are referred to as F1, C1, P1, IWT, F2, and C2. The sites of interest consist of free-standing eyewash stations and combination eyewash and shower stations, shown in figures 1 and 2.

Figure 1: Free standing eyewash station

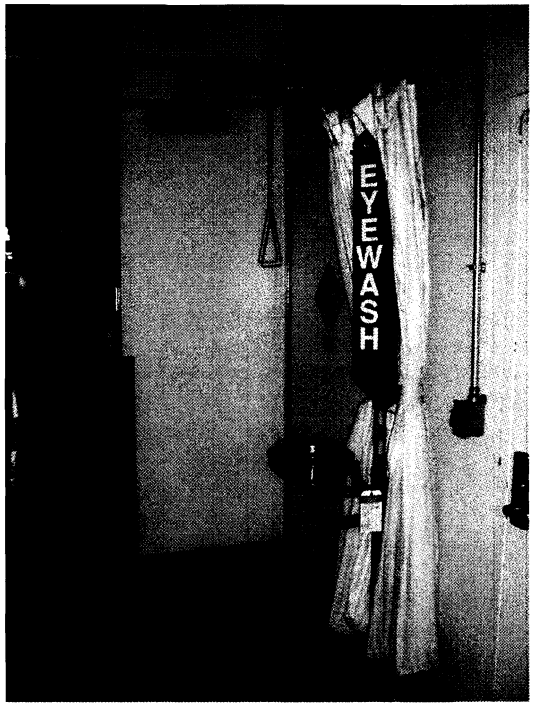

Figure 2: Combination eyewash and shower station

Most of the eyewash stations, whether free-standing or in combination, also serve as eye/face washes. Close-up shots of these can be seen in figures 3 and 4.

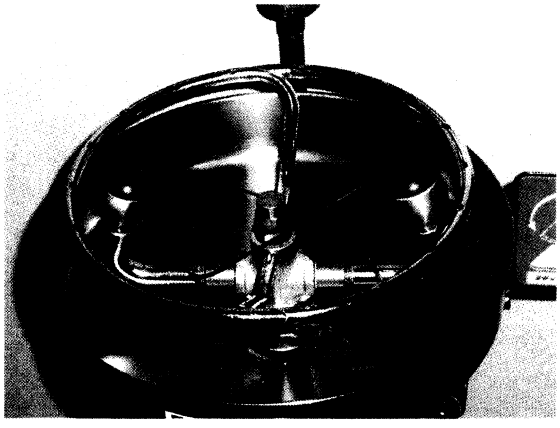

Figure 3: Free-standing eye and face wash

Figure 4: Free-standing eyewash

Some of the combination eyewash and shower stations also have an attached hose, drawing on the same source of flushing fluid, as seen in figure 5.

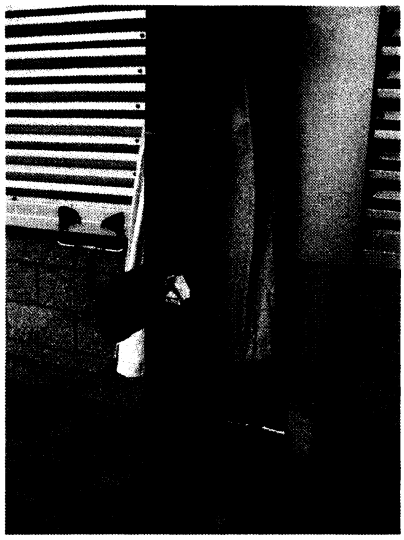

Figure 5: Combination station with hose

Chlorinated water is supplied by the local county water treatment facility. The potable water from the treatment facility is pumped directly to all of the emergency eyewash and combination eyewash and shower stations, and no further treatment or filtering occurs prior to reaching these stations. The F1, C1, P1, and IWT building piping networks run on a recirculating line with the general tap water within each of these buildings. This ensures constant recirculation of the potable water; however it does not correct for piping extensions, which create dead zones not included in this pattern. The F2 and C2 buildings do not share this recirculation distinction.

Each of the F1, F2, and IWT buildings houses external combination eyewash and shower stations. All six of the buildings house internal eyewash stations and combination eyewash and shower stations. External stations are located outside along the perimeters of each of the buildings, which expose them to the elements. Internal stations are contained within controlled environments and are not exposed to the elements. All of the external stations are flushed weekly and all of the internal stations are flushed monthly.

During flushing activities, water is allowed to flush from each outlet for about one minute. External stations are electrically heat traced to protect the piping from freezing, not to create tepid water for user comfort.

The F1 building houses a total of 86 stations including free-standing eyewash stations and combination eyewash and shower stations. The F2 building houses a total of 118 stations. The P1 building houses a total of 24 stations. The IWT building houses a total of 16 combination eyewash and shower stations. The C1 and C2 buildings house a total of 6 and 7 combination eyewash and shower stations, respectively.

Sampling Sites

Between 10-15% of the stations housed at each building were randomly chosen to represent the population of free-standing eyewash stations and/or combination eyewash and shower stations within each building. This ensured equal representation of the total population of stations among the buildings. Randomness was determined by listing all stations on lined paper, respective to each building, and then utilizing a table of random digits to choose the lines. There was no preferential treatment over sampling from the free-standing eyewash stations and combination eyewash and shower stations, as the treatment and source of water are from the same municipal supply, and both have similar potential for dead zones. The collection of samples from the eyewash or shower within the combination stations was random, as both were supplied by a single source of flushing fluid.

Collection Methods

The preferred collection method for proper *Legionella* analysis is through bulk water sampling, however, swab sampling is also a valid collection method.[17] For this study, the preferred method for collecting biofilm and detecting other bacteria was via water and swab sampling. When possible, control water samples from frequently used sources and blank swab samples accompanied the research samples. These control samples served to determine if contamination was prevented efficiently during sampling, and to determine the sterility of the sampling media.

Swab samples for the analysis of both *Legionella* and other bacterial organisms were collected from all of the buildings. Sterile transport swabs (Healthlink TransPorter, LQ Stuart 4432, 76A1 ex. 2006/12) were used to collect potential biofilm. Preparation of the swab for sampling was completed by removing the sealed cap to the empty swab vial, inserting a sterile swab into the vial, and moistening the swab tip with the vial's transport solution. Swab sampling was performed by swabbing the suspect area or material and replacing the swab back into the vial. Swab samples were collected prior to any initial water flow in order to capture potential undisturbed biofilm organisms. When possible, during the collection of biofilm from the eyewash stations, one of the two eyewash aerator outlets was removed and the piping directly leading to the water outlet was swabbed. The area swabbed is shown in figure 6.

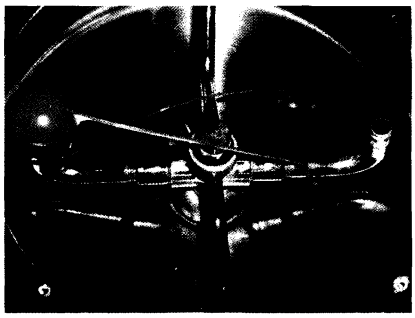

Figure 6: Swab area of piping

Alternative locations for the collection of biofilm from the eyewashes were sought for several of the stations when there was difficulty in removing the outlets. These locations included the swabbing of the surfaces inside the outlet cover, or the interior walls of the piping just before the double outlet piping split. These areas are shown in figures 7 and 8.

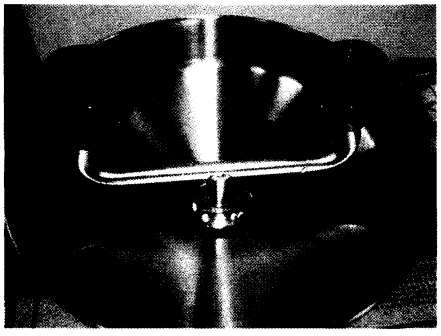

Figure 7: Swab area of outlet cover

Figure 8: Piping before outlet split

Biofilm collection from the showers included slipping the swab tip past one of the aerator outlets and swabbing the piping directly leading to the aerator. The area of the aerator outlets swabbed is shown in figure 9.

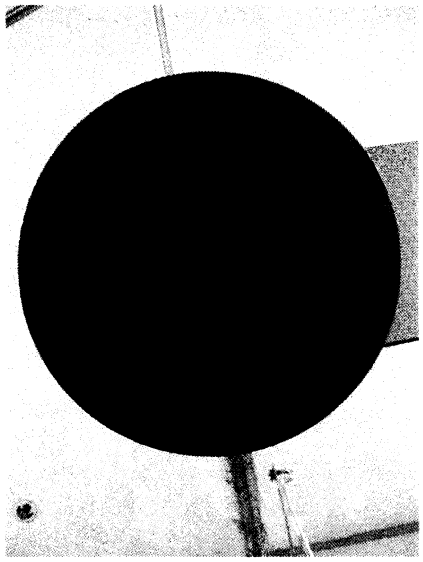

Figure 9: Shower aerator

Water samples for the analysis of *Legionella* were collected from all of the buildings, and only from the F1 and P1 buildings for the analysis of other bacterial organisms. These samples were collected using 250ml sterile PETG bottles (lot#538826, exp 10/28/09). Water collection was performed by removing the bottle cap, placing the bottle opening underneath the source outlet, and activating the station to catch the initial flow of water from the stream. This initial sample was intended to capture the level of contamination at the source outlet. In cases when the eyewash outlet was the collection source, the faucet opposite the one swabbed served as the water source. One bottle was used for the collection of water for *Legionella* analysis at each sampling location, with an approximate volume of 250ml per sample. One bottle was used for the collection of water for the analysis of other bacterial growth at each sampling location, with an approximate volume of 25ml per sample. The temperature of all water samples was taken immediately after collection using a Raytek Raynger MX2 Infrared Thermometer (serial number 221261-0101-0002, calibrated 7/2005). The purpose of the temperature

readings was to aid in analyzing the results by determining viable temperature ranges for any bacterial growth detected in the samples.

All samples were collected in or with their own individually labeled media. Nitrile gloves were used and replaced between sampling to prevent cross-contamination. Samples were refrigerated during intermittent periods within same-day sampling for the purpose of preventing temperature increases, which could have induced microbial growth. All shipped samples were received by the respective laboratory for analysis within 24 hours of sampling. The shipped samples were packed in insulated containers, with single-use icepacks, in such a manner as to prevent cross-contamination or spillage of the containers. The samples were protected from temperature extremes at all times, and the icepacks served to retard growth of any organisms. Hand-delivered samples were taken directly to the respective laboratory the same day sampling was performed.

Sample Collection

An initial collection round of representative water samples, including a few swab samples, for the detection of *Legionella* was conducted on three separate days during October 2005. These samples were collected to determine the temperature ranges during a neutrally temperate season, as well as to determine the extent of *Legionella* contamination, if any. The water samples were treated as non-potable water, because of the expectation of finding high levels of the organism. This treatment entailed a non-sensitive protocol for analysis to detect if high levels of contamination existed. If low or non-detectable levels existed, then the laboratory's more sensitive potable water protocol

was utilized. Non-detectable levels indicated levels that were below the laboratory's limit of detecting the organism based on the procedure utilized.

On the 10th of October, 3 swab and 10 water samples were collected from the F1 building. Of the water samples, 4 were collected from free-standing eyewash stations, and 6 were collected from the combination eyewash and shower stations. Four of these latter samples were from showers, while the remaining 2 were taken from the eyewashes. The temperatures of these water samples ranged from 19.9°C to 23°C, the recorded high temperature for that day was 22.2°C.[16] The swab samples were collected from an eyewash outlet and a shower outlet from a combination station and an eyewash outlet from a free-standing station. All of these samples were collected from internal stations.

On the 11th of October, 1 swab and 9 water samples were collected from the P1, IWT, C1, and C2 buildings. All of these samples were collected from the combination eyewash and shower stations. Seven of the water samples were from the eyewashes, 1 was from a shower, and 1 was from a hose. The temperatures of these water samples ranged from 18.1°C to 22.9°C. The recorded high temperature for that day was 20°C.[16] Two of the water samples were collected from external stations, with temperatures of 18.1°C and 18.4°C. The swab sample was taken from an eyewash outlet in a combination eyewash and shower station.

On the 14th of October, 2 swab and 14 water samples were collected from the F2 building. Of the water samples, 7 were collected from free-standing eyewash stations and 7 were collected from the combination eyewash and shower stations. Four of these latter samples were taken from showers, while the remaining 3 were from eyewashes.

The temperatures of these water samples ranged from 17°C to 22.8°C. The recorded high temperature for that day was 25°C.[16] The swab samples were taken from a free-standing eyewash station and an eyewash within a combination eyewash and shower station. All of these samples were collected from internal stations.

In late November and early December 2005, a second collection round of samples was taken from the P1, C1, C2, IWT, and F2 buildings. Water samples were collected for the detection of *Legionella*, and swab samples were collected for the detection of other bacterial organisms. Based on the non-detectable levels of *Legionella* found during the first round of sample collection, the water samples during this collection round were treated as potable water for the detection of low counts of *Legionella*. The standard procedure for the analysis of the swab samples for the other bacterial organisms maintained a high limit of detection for the small area swabbed. This included a non-sensitive protocol for analysis to detect if high levels of other bacteria existed. If low or non-detectable levels existed, then a more sensitive procedure to detect even lower counts of the other bacterial organisms could be utilized.

On the 28th of November, 8 water and swab samples were collected from the P1, IWT, C1, and C2 buildings. Of the water samples, 1 was collected from a free-standing eyewash station, and 7 were collected from combination eyewash and shower stations. Four of the latter samples were taken from the eyewashes, and the remaining 3 were taken from the showers. The temperatures of these water samples ranged from 18.3°C to 25.1°C. The recorded high temperature for that day was 23.3°C.[16] Two of the 8 water

samples were collected from external stations with temperatures of 22.8°C and 25.1°C. The swab samples were collected from the same locations as the water samples.

On the 2nd of December, 14 swab and water samples were collected from the F2 building. Of the water samples, 9 were collected from free-standing eyewash stations, and 5 were collected from combination eyewash and shower stations. Three of the latter samples were taken from eyewashes, while the remaining 2 were taken from showers. The temperatures of these water samples ranged from 18.4°C to 21.8°C. One of these samples was taken from an external station with a temperature of 18.4°C. The recorded high temperature for that day was 8.3°C.[16] The swab samples were collected from the same locations as the water samples. However, 3 of the swab samples had to be collected from the outlet covers, 1 from a free-standing eyewash station, and 2 from eyewashes within the combination stations.

A third collection round of samples taken from the F1 and P1 buildings in early December of 2005 was conducted. Water samples were collected for the analysis of both *Legionella* and other bacterial organisms. The water samples for *Legionella* analysis were treated as potable water for the detection of low counts of *Legionella*. A more sensitive procedure for the analysis of swab samples was utilized for the detection of low counts of other bacteria.

On the 9th of December, 5 water samples for *Legionella* analysis, and 5 water and swab samples for the analysis of other bacterial organisms were collected from the F1 building. Three of the 5 stations sampled were combination eyewash and shower stations, the remaining 2 were from free-standing eyewash stations. Of the combination eyewash

and shower stations, samples were collected from 2 showers, and 1 eyewash. The temperatures of these water samples ranged from 21.7°C to 22.9°C. All of these samples were collected from internal stations. The recorded high temperature for that day was 6.1°C.[16] The swab samples were collected from the same locations as the water samples; however, the 1 swab sample from the eyewash within the combination station was taken from the main pipe just before the split to both eye faucets.

On the 12th of December, 7 water samples for *Legionella* analysis and 7 water and swab samples for the analysis of other bacterial organisms were collected from the F1 and P1 buildings. Four of the 7 stations sampled were free-standing eyewash stations, and the remaining 3 were combination eyewash and shower stations. Of the combination eyewash and shower stations, samples were collected from 1 eyewash and 2 showers. The temperatures of these water samples ranged from 16.4°C to 21.6°C. All of these samples were collected from internal stations. The recorded high temperature for that day was 9.4°C.[16] The swab samples were collected from the same locations as the water samples.

Laboratory Analysis

Two separate American Industrial Hygiene Association (AIHA) accredited microbiological laboratories were utilized for sample analysis. One laboratory was utilized for its specialty in the analysis of *Legionella* bacteria, while the other laboratory was utilized for analyses of general bacterial organisms. Culture analyses for both *Legionella* and other bacterial organisms were utilized to determine viable bacterial counts. A direct fluorescent antibody (DFA) conjugate test by which the bacterium

fluoresces when viewed microscopically, and a polymerase chain reaction (PCR) method, which amplifies DNA for detection, are useful methods in determining presence of bacteria. Yet both methods are prone to false negatives and false positives, as there is no discrepancy among viable and non-viable organisms.[17,22] Thus, the DFA test or PCR method should be used as a supplement to the culture method, not as an alternate detection means. The DFA test is included as standard procedure in the laboratory analysis for *Legionella*, whereas PCR is an additional analysis, therefore DFA was used in conjunction with the *Legionella* culture method in this study. As it is unknown what bacteria will be found during the analysis for other bacterial organisms, the culture method was solely employed for their detection.

Upon receipt of the *Legionella* samples by the respective laboratory, they were prepped for the appropriate procedure for analysis, and analyzed for the detection of *Legionella pneumophila* sero groups 1-6. All water samples considered as potable water were filtered, utilizing a separate filter per sample, and then the filters were vortexed in sterile water. Prior to filtering the samples, it was up to the discretion of the laboratory technician to acid-treat suspected dirty water samples to clear them of other contaminating bacteria. All of the non-potable water samples were first cleared of other contaminating bacteria with an acid-treatment, and then diluted with sterile water. Culture plates were then inoculated with aliquots of 100µl of the resulting suspensions, for each process. Laboratories utilize aliquots so that remaining original samples could be used for quality control verification. The potable water protocol used the entire amount of the original sample, which was filtered and resuspended, and the non-potable

water protocol used 1ml of the original sample. All swab samples were placed in a buffered solution, which then had aliquots cultured. Select media was required for the culture of *Legionella* and consisted of buffered charcoal yeast extract (BCYE) agar plates, on supplemented (with antibiotics) and unsupplemented plates. The media were then incubated for up to ten days at 35°C. Negative results were reported on the tenth day, however, suspect colonies were further isolated and confirmed positive or negative by the DFA test.

Upon laboratory receipt of the samples for the analysis of other bacterial organisms, they were prepped for the appropriate procedure for analysis. All water samples were inoculated directly onto culture plates with 1ml aliquots. The swab samples from the second collection round were placed in separate 99ml neutral buffer solution bottles and allowed to soak prior to culturing 1ml aliquots. The swab samples from the third collection round were placed in 10ml vials of sterile water and allowed to soak prior to culturing 1ml aliquots. The culture media for the detection of other bacteria consisted of Blood and Maconkey Agar plates, which were incubated at 35°C for a minimum of three days, the normal growth period for bacteria. All growth was reported in colony forming units (CFU).

Chapter 3 Results

All samples for the analysis of *Legionella* resulted in non-detectable levels of the bacteria. The detection limits per milliliter for these samples ranged from less than 1 CFU to 5 CFU, and the detection limits per swab ranged from 10 CFU to 50 CFU.

Water samples for the detection of other bacterial organisms were collected from 12 free-standing eyewash and combination eyewash and shower stations. Swab samples for the detection of other bacterial organisms were collected from 34 free-standing eyewash and combination eyewash and shower stations. Results indicate that high counts of viable bacteria are contained within the water and biofilm substances (Table 1). None of the external stations resulted in detectable levels of bacteria.

Of the 8 swab samples submitted on the 28th of November, only 1 sample had detectable levels of bacterial organisms. The bacteria found were *Flavobacterium odoratum* and *Sphingomonas paucimobilis*. None of the 14 swab samples submitted on the 2nd of December contained detectable levels of bacterial organisms. For both dates the laboratory limit of detection was 1000 CFU/sq.in.

All 5 of the water samples submitted on the 9th of December resulted in detectable levels of bacteria. The bacteria found were *Staphylococcus*, *Moraxella*, and *Micrococcus* species. The laboratory limit of detection for these samples was 25 CFU/ml. Only 3 of the 5 swab samples submitted on this date resulted in detectable levels of bacteria. The

bacteria found were *Staphylococcus* and *Micrococcus* species, and *Flavobacterium odoratum*. The laboratory limit of detection for these samples was 1000 CFU/sq.in.

Table 1: Results for the detection of other bacterial organisms

Building	Sample #	Results		Free-standing Eyewashes	Combination Stations	
		Water cfu/ml	Swab cfu/sq.in.		Eyewashes	Showers
F1	1	7500	60000		*Staphylococcus*	
	2	1250	NBD			*Micrococcus*
		150	NBD			*Moraxella*
	3	1000	6000	*Micrococcus*		
	4	375	NBD			*Micrococcus*
	5	50	32000	*Micrococcus*		
		NBD	13000	*F. odoratum*		
	3	1175	100000	*B. pickettii*		
		325	NBD	*Staphylococcus*		
	4	1100	NBD		*Staphylococcus*	
	5	NBD	NBD			Sampled
	6	725	NBD	*Moraxella*		
		25	NBD	*Staphylococcus*		
	7	2500	NBD	*B. pickettii*		
		NBD	12000	*Staphylococcus*		
P1	1 - 2	N/A	NBD	1 Sampled	1 Sampled	
	1	7500	NBD			*B. pickettii*
	2	7500	NBD			*B. pickettii*
		NBD	130000			*Staphylococcus*
C1	3	N/A	NBD		Sampled	
	4	N/A	6000		*F. odoratum*	
		N/A	2000		*S. paucimobilis*	
IWT	5 - 6	N/A	NBD			2 Sampled
C2	7 -8	N/A	NBD		1 Sampled	1 Sampled
F2	1-14	N/A	NBD	9 Sampled	3 Sampled	2 Sampled

Note: NBD indicates No Bacteria Detected in the results column. "Sampled" indicates station tested with no detectable results. Bold border indicates different sampling dates within building. N/A indicates water sampling not performed.

Six of the 7 water samples submitted on the 12th of December resulted in detectable levels of bacteria. The bacteria found were *Burkholderia pickettii*, and *Staphylococcus* and *Moraxella* species. The laboratory limit of detection for these samples was 25 CFU/ml. Only 3 of the 7 swab samples submitted on this date resulted in

detectable levels of bacteria. The bacteria found were *Staphylococcus* species and *Burkholderia pickettii*. The laboratory limit of detection for these samples was 1000 CFU/sq.in.

Chapter 4 Discussion

It is of great value to know that there is no detectable *Legionella* colonization of the emergency eyewash and shower distribution systems at the industrial facility tested. This indicates little risk of exposure to and subsequent infection from the *L. pneumophila* bacterium that can be expected for users of these systems. The hypothesis of whether these stations serve as a significant source of the *Legionella* bacteria was found to support that they do not serve as a significant source of the bacterium. As there was ample nutrient source available to support growth of *Legionella*, and any residual chlorine within the water was not sufficient enough to prevent growth of the other, more susceptible, bacteria, it is suspected that the routine maintenance of these stations is the chief cause for the lack of *Legionella* detected. In the 1990 study by Paszko-Kolva et al. temperature measurements were not included, but mention of water standing in pipes at room temperature indicated that no heat treatment of the water was in effect at the time of the study.[18] This observation further supports the conclusion that the absence of detectable *Legionella* can be attributed to the maintenance regimen, as the water collected in this study primarily was maintained at ambient temperatures.

Nevertheless, microbiological analyses have limitations in detecting *Legionella* as the bacterium may be harbored and amplified within cells of protozoa or within a biofilm layer, and not be revealed during analysis.[14] This incident can result in a false negative

test result for *Legionella* when in fact *Legionella* may be present. Failure to detect the bacterium in any of the samples presents uncertainty during interpretation of results as unfavorable environmental conditions may have induced the *Legionella* bacteria into a dormant and nonculturable, but viable, state.

As a biofilm layer presents an ample nutrient source, amplification of *Legionella* bacteria and/or its supportive protozoan host may occur. Based on a suggestive correlation by the Cooling Technology Institute, when results have low bulk water *Legionella* counts and high biofilm counts of other bacterial organisms, a low immediate health risk may exist, but the potential for future problems cannot be ignored.[8] The alarming discovery in this study of the amount and variation of other bacterial organisms present in many of the samples is not only indicative of an ample nutrient source for the *Legionella* bacteria, but also another potential health hazard for users of these stations.

The pathogenicity of the other bacterial organisms found is minimal. *Sphingomonas paucimobilis* has been reported to cause respiratory infections, albeit infrequently, but also has limited virulence compared to other genera that cause similar infections. *Flavobacterium* species have been implicated as a cause of pneumonia. There is insufficient data available on the remaining bacteria as agents that cause respiratory illnesses. Similarly, for all of these other bacterial organisms, there is limited data implicating them as agents that cause eye infections. Essentially, the other bacterial organisms found in this study are either current inhabitants of the human body, only acting as opportunistic pathogens, and pose no health concerns under normal circumstances, or subsequent infections and diseases due to exposure to these organisms

are treatable. Primarily only immunosuppressed hosts and untreated injuries are at risk for disease and infection.

This study could not verify seasonal variability for contamination of *Legionella* as all samples were taken in the fall. However, it is suspected that such variability would affect only the external stations as all internal stations are maintained within a relatively constant temperature range. Increased water temperatures in the external stations, which may occur in warmer climates or seasons, could end the dormancy phase of *Legionella* and lead to its multiplication if contained within the water.

Future Research

This study offers valuable information on the security and potential hazards of using safety equipment. It provides much needed information as an exposure assessment on the use of emergency eyewash and shower stations that, although maintained regularly, are not used frequently, and therefore may pose a hazard for *Legionella* exposure. Future investigational studies might focus more on molecular techniques for the detection of *Legionella* presence and also on the detection and levels of protozoa present to find an association with the detection and levels of the *Legionella* bacteria. The incorporation of investigating various maintenance regimens and the detection of *Legionella* may be valuable in future studies in order to determine the minimum level of maintenance needed to sustain low or non-detectable levels of *Legionella.* Also of interest may be a study on those water distribution systems that do supply tepid or warm water to emergency eyewash and shower stations to determine the extent of *Legionella* and other bacterial colonization. One final suggestion for further examination of the potential

hazards of these stations is to determine the extent of other bacterial organism contaminations and their health implications.

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