

THE PRESENCE OF LEGIONELLAE IN RESPIRATORY DEVICES:
CONVENIENCE SAMPLING OF IDAHO LONG TERM CARE FACILITIES

by

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A thesis

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of the requirements for the degree of
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Sampling of Idaho Long Term Care Facilities

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The following individuals read and discussed the thesis submitted by student Andrew Nutting, and they evaluated their presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

My family, friends, thesis committee, colleagues, Boise State's Department of Community and Environmental Health, those in the Idaho Department of Public Health Public Health Division, and Idaho Bureau of Laboratories.

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ABSTRACT

Legionella is a bacterium found naturally in moist environments. Persons can become infected when they inhale airborne droplets of water containing such bacteria. Legionellosis cases associated with the use of respiratory devices such as Continuous Positive Airway Pressure (CPAP) units, jet nebulizers, portable room humidifiers, and respiratory ventilation equipment have been identified in context of a Legionellosis outbreak. However, a systematic search for the presence of *Legionella* bacteria in respiratory devices outside of a Legionellosis outbreak has not been reported. The goal of this study was to carry out such a survey on different types of respiratory devices in long term care facilities. Twenty-four respiratory devices including 9 CPAP, 4 BIPAPs, 5 oxygen humidifiers, and 6 ventilators were included in this study. A total of 72 sampling swabs were obtained for the testing of *Legionella* bacteria inside the respiratory devices. Culture and PCR tests for *Legionella pneumophelia* were made in tandem. No *legionella pneumophelia* bacteria were found in any of the respiratory devices sampled. Although there have been reports in the past of potential legionellosis cases associated with the use of respiratory devices, our data suggest that there are probably no legionella bacteria present inside respiratory devices separate of a Legionellosis outbreak.

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LIST OF ABBREVIATIONS

BiPAP	Bi-level Positive Airway Pressure
BSU	Boise State University
CDC	Center for Disease Control and Prevention
CPAP	Continuous Positive Airway Pressure
GC	Graduate College
IBL	Idaho Bureau of Laboratories
IDHW	Idaho Department of Health and Welfare
IDPH	Idaho Department of Public Health
LTCF	Long Term Care Facility
PCR	Polymerase chain reaction
TDC	Thesis and Dissertation Coordinator

CHAPTER 1: INTRODUCTION

Background

Legionella is a genetically diverse and ubiquitous bacterium that typically thrives in warm water between 50-70° C (Center for Disease Control, 2016). The name of the legionella bacteria and one of the illnesses associated with it (Legionnaires' disease) is derived from an outbreak that occurred during an American Legion convention in Philadelphia in 1976 (CDC, 2016). The bacteria are typically found in stagnant water pipes, showerheads, Jacuzzis, and cooling towers of commercial heating, ventilation, and conditioning units (CDC, 2016).

Legionellosis cases are generally found in individuals that have been exposed to aerosolized water droplets from fountains, commercial heating, ventilation, and conditioning (HVAC) units, and contaminated water (CDC, 2016). However, reports of legionellosis cases associated with respiratory devices in the literature is sparse. While cases in Idaho possibly associated with these devices have been reported, environmental sampling for legionellae in sporadic cases of legionellosis is not recommended by the Centers for Disease Control and Prevention (CDC). Outbreaks of legionellosis in long-term care facilities have also been reported in the literature, but the sampling of respiratory devices during outbreak investigation has infrequently been described, and no survey of respiratory devices in the absence of legionellosis cases has been reported.

Statement of the Problem

While previous research has focused on legionellosis cases during outbreaks, relatively little is known about the presence of legionellae in respiratory devices. In general, there is no literature on the association between the presence of legionella in respiratory devices leading to legionellosis. Furthermore, *Legionella* is an opportunistic bacterium and those who may be immunocompromised or have decrease immune response, such as the elderly, may be an increased risk for legionellosis (CDC, 2016). There may even more of a risk of infection to those who may be elderly and be on a respiratory device.

Purpose of the Study

The purpose of this study was to investigate the presence of legionellae in respiratory devices used in long term care facilities. The independent variables included the device type, specific areas on the device, time sampled, and duration of device use. The dependent variable was the of presence of legionella.

Research Questions

RQ1: Are legionellae present in respiratory devices found in regional long-term care facilities outside of an outbreak?

RQ2: Is there a difference in the presence of legionella between the different types, makes, and models of respiratory devices?

RQ3: Is there a difference in area swabbed on the device and presence of legionella?

Summary

Currently, there is limited information available regarding the presence of legionella bacteria residing inside respiratory devices. Few studies addressed the risk of legionellosis associated with the use of respiratory devices outside of an actual outbreak. The CDC does not recommend sampling for legionella outside of an outbreak. This makes it difficult to interpret results during an actual outbreak.

CHAPTER TWO: REVIEW OF LITERATURE

Legionellae

According to the CDC (2016) legionella bacteria's primary mode of transmission is through the water it thrives in, spreading through aerosolized water droplets that are eventually inhaled. The bacteria are ubiquitous in the environment and some studies have noted its prevalence (Kuroki et al., 2017 & Lau et al., 2013) It is also possible to become infected through aspiration while drinking water (CDC, 2016). It is unlikely for infections caused by legionellae to be transmitted from human to human.

Legionellosis

Legionellosis is a collective term to describe diseases that stem specifically from legionella bacteria. Legionellosis includes the two primary diseases Legionnaires' disease and Pontiac fever. Legionnaires' disease presents as a serious flu-like illness and can be fatal in those who have weakened immune systems, such as the elderly or infants. Pontiac fever typically presents itself as mild flu like symptoms with fever and muscle aches (Fields et al., 2002).

Research suggests the legionellosis cases caused by legionella are typically associated with a disruption of the biofilm the legionella lives in as the bacteria grows in colonies (Inglis et al., 1989). Disruption of the biofilm liberates the colonies of legionella into the surrounding environment, where they are then aerosolized and inhaled, potentially infecting a person. While there are currently more than 40 known species of legionellae bacteria, not all species of legionellae can cause legionellosis; *L.*

pneumophila (legionella pneumophila) serogroups 1, 3, 4, and 6 cause most human infections (Fields et al., 2002).

Faris et al. (2005) described a few instances in the literature where legionellosis was caused by *L. pneumophila* serogroup 13. They noted that these cases of legionellosis from serogroup 13 were found in a wide geographic area that included Australia, Scotland, and New Zealand, which was unusual because such cases caused by serogroup 13 are rarely found in humans.

Most cases described in the literature were confirmed by culture and direct fluorescent antibody staining or by indirect immunofluorescent antibody tests (Faris et al. 2005). However, “Most diagnostic tests are directed at the species that causes most of the reported human cases of legionellosis, *L. pneumophila* serogroup 1. Therefore, information attributable to other species and serogroups of legionellae is lacking” (Fields et al., 2002). Due to the ubiquity of legionella bacteria, the CDC (2016) does not recommend sampling outside of an active outbreak. Therefore, little is known on the relationship of legionella presence in respiratory devices outside of an outbreak.

Transmission of Diseases through devices

A review of the literature has not yielded sufficient information about confirmed cases of other bacterial transmission through respiratory devices. A study focusing on CPAPs by Chin et al. (2013), suggests that although these devices are frequently found to be colonized by bacteria, there does not appear to be any association between bacterial colonization and chronic rhinosinusitis.

The presence of legionella in respiratory devices such as; CPAPs, BiPAPs, humidifiers, and ventilators, has not been investigated previously. Two studies reported

“probable transmission” through a contaminated respiratory device (Stephens et al., 2015 & Arnow et al., 1982). Two more studies concluded direct cases of legionellosis caused by a CPAP devices (Schnirman et al., 2017 & Srivali et al., 2013). Many of the devices cited in the literature were CPAPs owned by individuals of confirmed legionellosis cases. The probable transmission of legionella was attributed to dirty CPAP machines for treating individuals suffering from obstructive sleep apnea.

Chapter II Summary

Legionella is a ubiquitous bacterium that thrives in warm and wet environments, such as indoor water pipes, jacuzzies, and HVAC cooling towers. While there are more than 40 known species of legionella and even more serotypes. *Legionella pneumophila* serogroups 1, 3, 4, and 6 cause most human infections. Legionella bacteria can cause Legionnaires’ disease and Pontiac Fever. The latter of the two is relatively mild and most patients make a full recovery. The former may be fatal, for those who are immunocompromised. Due to the ubiquity of the legionella bacteria in the environment, the CDC does not recommend sampling for legionella bacteria outside of an outbreak. Since the existing literature is primarily based on data for outbreak events, data presented cases make it hard to generalize (Phin et al., 2014).

CHAPTER THREE: METHODS

Overview

The current study was designed to investigate the presence of legionella bacteria inside respiratory devices used in long-term care facilities. Legionellosis outbreaks linked to continuous positive airway pressure (CPAP) and other respiratory devices (e.g., jet nebulizer, portable room humidifier, respiratory ventilation equipment) have been reported in the literature, however, little is known about the presence of legionella contamination outside of an outbreak. Furthermore, while known cases in Idaho possibly associated with these devices have been reported, little evidence has been gathered since environmental sampling for legionellae in sporadic cases of legionellosis is not recommended by the Centers for Disease Control and Prevention (CDC). While outbreaks of legionellosis in long-term care facilities (LTCFs) have been reported in the literature, no surveys of respiratory devices in the absence of legionellosis cases has been reported.

Participants

Data were collected on 24 conveniently sampled respiratory devices (9 CPAP, 4 BIPAPs, 5 oxygen humidifiers, and 6 ventilators) found in 4 long term care facilities located in the northwestern U.S. The owner of the device, whether facility or resident, voluntarily consented for the device to be sampled and no specific data were collected on any human subject.

Respiratory Devices.

Respiratory devices as defined in this study include Continuous Positive Airway Pressure (CPAP), Bilevel Positive Airway Pressure (BiPAP), jet nebulizer, portable room humidifier, respiratory ventilation, and any other personal use devices used to humidify ambient air. These devices are prescribed to those who may have impaired respiratory function and are in need of assistance during inhalation or exhalation by mechanical means. These devices humidify ambient air and provide supplemental oxygen to the user when needed.

CPAPs & BiPAPs

Continuous Positive Airway Pressure (CPAP) devices and Bi-level Positive Airway Pressure (BiPAP) devices are usually prescribed to treat sleep apnea. CPAPs provide continuous airway pressure on resident's during inhalation when they sleep. BiPAPs differ from CPAPs by providing additional levels of airway pressure during exhalation. Picture 2 is a representation of typical CPAPs found at the facilities.

Humidifiers

Humidifiers are devices that add moisture to supplemental oxygen. The increased humidity prevents any discomfort associated with continuous dry air. Picture 3 is a representation of a typical humidifier found at the facilities.

Ventilators

Ventilators are devices that provide a high flow oxygen therapy. Some devices heat the water to 37 degrees Celsius before the air is passed through it to ensure the gasses are adequately humidified for user comfort. Picture 3 is a representation of a typical ventilator found at the facilities.

Materials

Approximately 72 Puritan EnviroMax: Sterile Environment Sampling Swabs (REF: 25-88010 PF) were provided by Idaho Bureau of Laboratories for swabbing the respiratory devices, extra swabs were provided as needed. Consent forms (see Appendix A), as well as information packets, were provided to address potential concerns expressed by either facility administrators or device owners. Packets included basic project and contact information and information about the project coordinator and primary intern (see Appendix B).

Sample

Initial meeting with facilities was set up to gain consent from administrators to come in and sample potential devices. Once a rapport was established, a time was scheduled for sampling. Devices were included in this study if they were being used at the facility and consent provided by the device owner. Sampling was carried out over a period of seven months; samples were collected from five randomly chosen devices, and three samples were collected from each machine. Due to capacity constraints placed on Idaho Bureau of Labs where identification of the legionella bacteria samples occurred. Only fifteen samples from five devices were sampled and tested.

Protocol development

Swabbing for legionella on various areas on the device; tubes, face masks, filters, and external surfaces, and testing the water reservoir were found to be an effective way of sampling for legionella bacteria as reported in the literature. In the study by Inglis et. al, the swabbing method included the inner surface 1-cm on each end, the outer surface at the lower cuff margin, and the tip of every tracheal tube (1989). Much of the literature

reported a similar methodology where swabbing was performed on either the outlet inner port, blower hole, outer machine surface, reservoir, face mask, inlet filter housing, and inlet filter.

The method of swabbing replicated best practices used for environmental sampling assuring even and consistent area swabbing when sampling in order to achieve a density of bacteria that could be tracked after a culture test was completed. However, the areas swabbed were not always consistent and the ability to back track the concentration of bacteria was not possible.

Consent forms

Consent forms were created for both the facility and device owners. An information packet was also developed to provide participants an understanding of the project methods and goals. Also, participants were informed that their involvement was voluntary and that they could leave the project at any time. (See Appendix B)

Data collection tool

A data sheet was developed to document variables such as duration of time machines were used, the date a machine was cleaned, the date a machine was wiped down...etc. (See Appendix A).

Procedures

First the researchers met with facility administrators to establish rapport and get consent from the facility to conduct the research study; agreement to participate was voluntary. Then the researcher worked with facility staff to determine respiratory device cleaning schedules and established best times for devices to be sampled. A list of devices was sent to the researcher before the sample date. A randomly selected group of 6 or

more devices was created, which allowed for other devices to be sampled from in the event a device was no longer in use. Unique identifiers were created for each sample tube and marked ahead of time. This was done to expedite the process and the location of swab site codes that were marked when it was determined which site of the device was sampled.

On the scheduled sample date, the researchers met with designated facility staff to be accompanied throughout the sampling process. After brief introduction and explanation of the project was given to the resident researchers provided information packets to the device owners that outlined the overall project and gave contact information in the event any of the owners had questions. Researchers then asked through either verbal or written consent from the owner of the device (signatures from two witnesses were used for verbal consent), whether it be the facility or resident, to sample the device. Signatures from two parties, usually a researcher and an employee, were used to show witness that verbal consent was given. Once consent was given the researchers continued with the sampling process. As one researcher sampled the device, the other researcher asked questions from the data collection tool to gather information pertaining to the device. As each area on the device was swabbed the researcher gathering information for the collection tool marked the appropriate code at the end of the identifier on the tube and stored it away for safe keeping. This process was done until a total of 5 devices were completed, 15 swabs, or until there were no more devices to be sampled from.

After sampling, the researcher then filled out a request form to be submitted to IBL for testing. The request form contained all the unique identifiers of each sample tube.

The request form and all the samples were submitted to IBL for testing. This process was completed until all the facilities that initially agreed to participate were sampled. Lab results were usually available in two weeks and were posted to a secure cloud-based work drive.

Testing

Typically testing relies on urinary antigen tests to diagnose legionellosis, however this was not feasible in this study. This study utilized culture tests accompanied by a polymerase chain reaction test (PCR) to confirm the presence of legionella bacteria.

Methodology

Review of the literature suggests that two kinds of test be run for determining the presence of legionella (Anvi et al, 2016 & Shen et al, 2006). Therefore, culture and PCR tests were conducted in tandem for each sample. Culture tests were used to help confirm the presence of live legionella bacteria as well as potentially being able to calculate the possible concentration. PCR helps confirm the presence of legionella as well as the species.

Variables

Independent variables included make and model of the device, the area swabbed on the device, the time it was swabbed, the time of last use, frequency of use, and duration of time since it was last cleaned. The presence of legionellae is the dependent variable and was a binomial, yes/no variable.

CHAPTER 4: FINDINGS

There were no legionella bacteria found in culture testing and no positive results for *L. pneumophila* in the PCR test.

Analysis

Descriptive statistics of the duration of time since last cleaning, gathered from the data collection tool (appendix A), showed that devices went on average 10.6 days before being sampled from with a min of 1 day and a max of 35 days. The frequency of cleaning these devices varied highly and there is no standard method of cleaning these devices besides exchanging tubes and facemasks, where frequency is also dependent on various manufacturers recommendations.

Table 1 **Percentage of devices sampled**

<u>Device</u>	<u>Percentage</u>
CPAP	45.83%
BIPAP	16.67%
Ventilator	25.00%
Humidifier	0%
Oxygen Humidifier	20.83%

Table 2 Percentage of locations swabbed

<u>Location</u>	<u>Percentage</u>
Mask	15.28%
Distal Tube (DT)	30.56%
Reservoir (R)	27.78%
Proximal Tube (PT)	20.83%

Note. Proximity in reference to machine.

Table 3 Test results of each device swabbed

<u>Device</u>	<u>Positive</u>	<u>Negative</u>
CPAP	0	9
BIPAP	0	4
Ventilator	0	6
Humidifier	0	0
Oxygen Humidifier	0	5

CHAPTER 5: CONCLUSION

Discussion

There have been reports in the literature of potential legionellosis cases associated with the use of a respiratory device. Such cases have also been reported in Idaho. Our data suggests that there may not be any legionella bacteria present inside respiratory devices outside of an outbreak.

Limitations

Several limitations emerged during the study. The use of convenience sampling and small sample size affect the generalizability of the study's findings. Participation from LTCFs was voluntary and any facility that was willing to participate was sampled from. Only four out of seven LTCFs who were contacted agreed to participate and only three had devices actively in use, this could be due to the potential legal problems with finding legionellae in facility run respiratory devices. Three LTCFs had more than five machines to be sampled from (one machine yields three samples) therefore, a second sampling time would have to have been scheduled to sample the rest of the machines. However, a second appointment was not made available to all facilities given time constraints. Only two of the facilities were re-sampled from. There was a final total of 24 devices at the conclusion of sampling.

Furthermore, the Idaho Bureau of Laboratories was limited to only be able to process 15 samples per week. After the first round of devices were sampled, IBL reported that there was a positive PCR test for *legionella spp.* (legionella species). However, it is

still not known what specific species of legionella bacteria it was. It was initially thought that the swabs were contaminated but after further investigation by IBL it is possible that it was the reagents used during testing that were contaminated. After further testing by IBL it was concluded that the legionella was not coming from the actual devices but showing up in the background of the test. There was no perceived risk in proceeding with the study given that a majority of legionellosis cases are associated with *legionella pneumophila* and not any other species of legionella.

A further limitation was that halfway through the study there was an issue with the type of swabs being used. Initially, it was assumed that the swabs were pre-moistened with a solution that neutralizes strong sanitizers that may be used when cleaning respiratory devices as well as provide a favorable environment for any potential legionella bacteria that may be present. Instead, dry swabs were used. Due to the backorder of pre-moistened swabs, time constraints, and lack of viable alternatives, dry swabs continued to be used for the duration of the study. One rationale for the continued use of dry swabs was the moist environment of the devices was enough for any potential legionella bacteria to survive until it was tested. Also, one of the primary reasons a premoistened swab may be used is to neutralize any harsh disinfectants that may be lingering when swabbing various surfaces. This led to re-contacting the facility to ask whether disinfectants were used when cleaning respiratory devices and what the active ingredients were on the cleaning products. There were no harsh disinfectants being used while cleaning the respiratory devices, so there was little concern for lingering chemicals that may have created an unfavorable environment for the legionellae to survive in until testing.



Picture 1. Non-reusable reservoir



Picture 2. CPAP/ BiPAP



Picture 3. Ventilator/ humidifier unit

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APPENDIX A
Data Collection Tool

Data Collection Instrument for Legionella Project

Time: _____ **Date:** ____/____/____

Facility: _____

1. Is the Machine owned by the facility? (Y/N)?
 - If no, is the machine rented or owned by the resident?
 - o Has consent been verified? Check the box for Yes.
2. What kind of device is this? CPAP, BIPAP, respiratory ventilator, oxygen humidifier, room humidifier
3. What is the make of the machine?
4. What is the model of the machine?
5. What is the serial number of the machine?
6. Is there a specific protocol in place for cleaning the machine (Yes/No)?
7. How many days per week is the device used?
8. When was the last time this device was used?
9. When was the last time this device was cleaned (in days)?
10. When was the last time the device was wiped down (in days)?
11. When was the last time the tubes were cleaned (in days)?
12. When was the last time the tubes were replaced (in days)?
13. Is the tube a heated or non-heated tube?
14. When was the last time the reservoir was replaced/refilled (in days)?
15. When was the last time the mask was replaced?

Completed by:

APPENDIX B

Information Packets for Device Owner

Handout for Residents for Legionella Project

Hello! We are two students from Boise State University, Andrew Nutting and Jennifer Hoolehan, who are part of a project with the Idaho Division of Public Health to look for legionellae in respiratory devices (such as CPAPs and ventilators).

Project Background

Legionellae are bacteria that live in water like lakes and streams everywhere. If legionellae grow and spread in building water systems, they can cause pneumonia, especially in people who already have certain other health problems or who smoke. Pneumonia from legionellae in respiratory devices has been reported in the literature. Some cases of disease caused by legionellae that were possibly associated with respiratory devices have been seen in Idaho. We are looking to see if legionellae can be found in respiratory devices when no one has legionella disease to help understand what it means if legionellae are found in respiratory devices.

Q&A

“What are you testing?”

We are testing parts of respiratory devices that gather moisture or hold water. The Idaho Bureau of Laboratories will test for legionellae bacteria.

“Are there any medical risks associated with having legionella in a respiratory device?”

There are some reports of finding legionellae in devices used by people who had legionella pneumonia, but no one has looked for legionellae in devices used by people who don't have legionella pneumonia. We are doing this study to help answer this question.

“What will happen if a sample comes back positive?”

Our team will provide manufacturer's and CDC cleaning recommendations to your facility. After the machine is cleaned, we will retest it to verify that cleaning removed live legionellae.

Consent

By signing this form you acknowledge that you understand the outlines of the project and give your voluntary consent for us to sample your respiratory device. Whether you give permission or do not give permission will not affect any care or services you receive. We will give you the results of the test. If we find legionellae in your device,

it will undergo cleaning by the people responsible for device maintenance and we will retest it. You do not have to have your device tested and you can change your mind and decide not to participate at any time.

If you have any questions about the project, you may contact:

Andrew Nutting, Principal Investigator
Boise State University
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Dr. Kris Carter, Project Supervisor
Bureau of Communicable Disease Prevention, Idaho Division of Public Health
208-334-6674; kris.carter@dhw.idaho.gov

Name _____ Date _____

Signature _____

In the event written consent cannot be given,

Witness 1 of verbal consent

Name
and signature _____

—

Date _____

Witness 2 of verbal consent:

Name and signature

Date _____

Page Break

If you have any questions about the project, you may contact:

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Witness 1 of verbal consent

Name
and signature _____

—

Date _____

Witness 2 of verbal consent:

Name and signature

Date _____

What is the make of the device/machine? _____

What is the model of the device/machine? _____

What is the serial number of the device/machine? _____

APPENDIX C

Information Packets for Non-Residents

Consent form for owners who are not residents: Legionella Project

Project Background

Legionellae are bacteria that live in water like lakes and streams everywhere. If legionellae grow and spread in building water systems, they can cause pneumonia, especially in people who already have certain other health problems or who smoke. Pneumonia from legionellae in respiratory devices has been reported in the literature. Some cases of disease caused by legionellae that were possibly associated with respiratory devices have been seen in Idaho. We are looking to see if legionellae can be found in respiratory devices when no one has legionella disease to help understand what it means if legionellae are found in respiratory devices.

“What are you testing?”

We are testing parts of respiratory devices that gather moisture or hold water. The Idaho Bureau of Laboratories will test for legionellae bacteria.

“Are there any medical risks associated with having legionella in a respiratory device?”

There are some reports of finding legionellae in devices used by people who had legionella pneumonia, but no one has looked for legionellae in devices used by people who don't have legionella pneumonia. We are doing this study to help answer this question.

“What will happen if a sample comes back positive?”

Our team will provide manufacturer's and CDC cleaning recommendations to you and the facility where the device is being used. After the device is cleaned, we will retest it to verify that cleaning removed live legionellae.

Page Break
Consent

By signing this form you acknowledge that you understand the outlines of the project and give your voluntary consent for us to sample your respiratory device(s). Whether you give permission or do not give permission has no relation to any contractual services you have with device users or facility owners. We will give you the results of the test. If we find legionellae in your device, it will undergo cleaning by the people responsible for device maintenance and we will retest it. You do not have to have your device tested and you can change your mind and decide not to participate at any time.

If you have any questions about the project, you may contact:

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Name _____ Date _____

Signature _____

Make of the device/machine? _____

Model of the device/machine? _____

Serial number of device/machine? _____

