

COOLING TECHNOLOGY INSTITUTE

Legionellosis Guideline

Practices to Reduce the Risk of Legionellosis from Evaporative Heat Rejection Equipment Systems



September 2020

GDL-159(20)

This document was developed using the consensus process outlined in the CTI Operating Procedure 304
and has been approved for publication by the CTI Board of Directors

[COPYWRITED MATERIAL ALL RIGHTS RESERVED TO CTI](#)

Foreword

This Cooling Technology Institute (CTI) publication is published as an aid to evaporative heat rejection equipment purchasers, designers, and operators. This document may be used by anyone desiring to do so, and efforts have been made by CTI to assure the accuracy and reliability of the data contained herein. However, CTI makes no warranty of fitness for particular purpose or merchantability or any other warranty expressed, implied or statutory. In no event shall CTI be liable or responsible for Incidental, Consequential or Commercial losses or damage of any kind resulting from this publication's use; or violation of any federal, state, or municipal regulation with which this publication may conflict or for the infringement of any patent resulting from the use of this publication.

All CTI codes, standards, and guidelines are copyrighted with all rights reserved to CTI. The reproduction of any part of this or any other CTI code or standard is a violation of Federal Law. One must recognize and appreciate commitment by a number of volunteer members who donate their time to develop and update CTI codes, standards, and guidelines. The costs for code development and review, administrative staff support, and publication are essential and constitute a substantial expenditure on the part of CTI. The purchase price of these documents helps to offset these costs. Reproduction and distribution by others, whether in paper or electronic form, in addition to being unethical, undermines this system and constitutes a further financial burden on CTI. When copies are needed, you are requested to visit the CTI website at www.cti.org or call or write the Cooling Technology Institute, P.O. Box 73383, Houston, Texas 77273, (281) 583-4087. Please encourage your associates to purchase the required codes, standards, and guidelines rather than copy them. Your cooperation and understanding in this matter are greatly appreciated.

Nothing contained herein is to be construed as granting any right for the manufacture, sale, or use in connection with any method, apparatus, or product covered by letters patent, nor as insuring anyone against liability for infringement of letters patent.

This guideline document summarizes the best current state of knowledge regarding the specific subject and was developed using the consensus process outlined in CTI Operating Procedure 304 (OP-304). As such, this document represents a consensus of those individuals who have developed and reviewed this document, its scope, and provisions. It is intended to aid all users or potential users of evaporative heat rejection equipment.

Approved by the CTI Executive Board



Approved by the CTI Executive Board

This document has been reviewed and approved as part of CTI's Five Year Review Cycle.
This document is again subject to review in 2025

COPYWRITED MATERIAL ALL RIGHTS RESERVED TO CTI

TABLE OF CONTENTS

Section / Title	Page
1. PURPOSE	4
2. SCOPE	4
3. WHAT IS LEGIONNAIRE’S DISEASE	4
4. SYMPTOMS OF LEGIONNAIRES’DISEASE	5
5. MICROBIOLOGY	6
6. ECOLOGY	7
7. PRACTICES AND RECOMMENDATIONS FOR REDUCING RISKS ASSOCIATED WITH LEGIONELLA	7
8. ROLES, RESPONSIBILITIES AND RECORD-KEEPING	17
9. EVAPORATIVE HEAT REJECTION EQUIPMENT AND SYSTEM RECOMMENDATIONS	18
10. GENERAL MAINTENANCE AND INSPECTIONS	20
11. CONFIRMATION	20
APPENDIX A	
A-1: COOLING SYSTEM SHUTDOWN AND STARTUP	22
A-2: LARGE INDUSTRIAL COOLING SYSTEM SHUTDOWN AND STARTUP GUIDELINES	23
A-3: EXAMPLE OFFLINE CLEANING AND CHEMICAL DISINFECTION FOR COOLING TOWERS	27
A-4: EXAMPLE ONLINE CLEANING AND CHEMICAL DISINFECTION FOR COOLING TOWERS	28
A-5: GUIDELINE FOR EMERGENCY DISINFECTION – AN EXAMPLE EMERGENCY DISINFECTION PROCEDURE	29
APPENDIX B: EXAMPLE SESSILE BACTERIA MONITORING TECHNIQUE	30
APPENDIX C: INSPECTIONS AND PHYSICAL MAINTENANCE	32
APPENDIX D: PROFICIENCY FOR LEGIONELLA TESTING – ENVIRONMENTAL LABORATORY ACCREDITATION PROGRAMS	34
APPENDIX E: ROUTINE TREATMENT PROCEDURES	35
APPENDIX F: ADDITIONAL INFORMATION SOURCES	38

Legionellosis Guideline: Practices to Reduce the Risk of Legionellosis from Evaporative Heat Rejection Equipment Systems

1. PURPOSE

1.1 The purpose of this guideline is to provide practices that reduce the risk of exposure to *Legionella* from evaporative heat rejection systems, specifically evaporative condensers, closed-circuit cooling towers, and open-circuit cooling towers.

Note: *The use of the term “cooling tower” in this guideline refers collectively to evaporative condensers, closed-circuit cooling towers, and open-circuit cooling towers, unless otherwise noted.*

1.2 The document is intended to provide necessary information for evaporative heat rejection equipment associated with building water systems to support the development of water management programs such as for ASHRAE Standard 188. It is also intended to provide necessary information for evaporative heat rejection equipment on industrial process and power plant systems, programs which are subject to different criteria, operating characteristics, and regulations.

2. SCOPE

2.1 This guideline provides specific environmental and operational guidelines that contribute to the safe operation of evaporative heat rejection systems, specifically to reduce the risk of occurrence of legionellosis.

2.2 This guideline applies to owners, managers, and operators of facilities that include evaporative heat rejection systems. This guideline is also intended to apply to those involved in design, construction, installation, commissioning, operation, maintenance and servicing of evaporative heat rejection systems.

2.3 This guideline applies, as noted within, to systems including, but not limited to, building air-conditioning, industrial process, and power plant systems.

3. WHAT IS LEGIONNAIRES’ DISEASE?

3.1 Following the 1976 American Legion Convention at the Bellevue Stratford Hotel in Philadelphia, 34 attendees died, and 221 people became ill from pneumonia caused by the bacterium *Legionella pneumophila*. Although not recognized at the time, *Legionella* is not a new microorganism. It has since been found in archived tissue samples at the US Centers for Disease Control and Prevention (CDC). These specimens were taken from persons with previously undiagnosed pneumonia-like illnesses.

3.2 This disease is known as Legionnaires’ disease (LD) when it progresses to pneumonia. It is a respiratory infection that can strike individuals exposed to *Legionella* bacteria, especially those with weakened immune systems. The majority of infections are caused by the species *Legionella pneumophila*; though other species have been known to cause disease. Most outbreaks of Legionnaires’ disease associated with evaporative heat rejection equipment are caused by *L. pneumophila*, serogroup 1. Infection results from inhaling airborne water droplets or mist containing viable *Legionella*, which are small enough to pass deep into the lungs and find their way to the alveoli, the small pockets in the lungs. Aspiration of water containing *Legionella* is also a transmission route to the lungs resulting in disease (Mercante 2015). The dose of *Legionella* required to infect humans is not definitively known. Normal ingestion of *Legionella* has not been shown to cause illness. However, aspiration during the ingestion process, such as may occur when

choking or spontaneously during the ingesting or swallowing process, can allow oral fluids and/or particles to by-pass natural gag reflexes and enter into the respiratory tract and lungs. Legionnaires' disease typically has an incubation period of two to ten days, with most people displaying symptoms within 5 days. Most reported cases have occurred in the over 50 year old age group. Although healthy individuals may develop Legionnaires' disease, people at increased risk of infection include smokers, patients with cancer, chronic respiratory diseases, diabetes, kidney disease, and any immunosuppressed condition. The CDC reports the overall mortality rate for Legionnaires' disease at approximately 10%; but with immunosuppressed persons, those with other underlying disease and conditions, and those that acquire the disease in the hospital (healthcare environment), this rate is 25% or greater.

3.3 *Legionella* are common freshwater bacteria, being detected in ground and surface water (lakes, ponds, rivers and streams) as well as in muds and some soils. The organism can survive typical chlorine disinfection for potable water and consequently appear in finished water distributed to homes, buildings and industry, including the water used for potable and non-potable use – such as humidifiers, spas, water features, ice machines and evaporative cooling equipment. *Legionella* can grow in biofilm or slime on surfaces in contact with water and can easily adapt to conditions within water distribution systems. *Legionella* bacteria grow in association with other microorganisms, most notably amoebae and other protozoans. The disease burden from Legionnaires' disease is significant. *Legionella* bacteria are among the top three causes of sporadic, community-acquired pneumonias (Neil and Berkelman; von Baum) and are a common cause of hospital-acquired (nosocomial) cases of pneumonia. Many disease cases can go undiagnosed while others are often treated with antibiotics without

looking for the underlying cause. The under-diagnosis can result in underreporting, so the true incidence of Legionnaires' disease can only be estimated. For instance, the CDC has estimated that the disease infects 10,000 to 15,000 persons annually in the United States. The U.S. Occupational Safety and Health Administration (OSHA) estimates that over 25,000 cases of the illness occur each year, causing more than 4,000 deaths. There is also a significant cost in terms of healthcare dollars, estimated to be between \$100 and \$300 million annually (Collier 2012). Legionnaires' disease was responsible for more than half of all drinking water outbreaks that occurred in building premise plumbing escaped detection except for the increased awareness of the disease.

4. SYMPTOMS OF LEGIONNAIRES' DISEASE

4.1 Initial symptoms of Legionnaires' disease include fever, chills, headache, and muscle pain. A dry cough soon develops and most patients suffer breathing difficulty. Some patients also develop diarrhea or vomiting and can become confused or delirious. Legionnaires' disease may not always be severe; in community outbreaks, mild cases may be recognized that would probably have escaped detection except for the increased awareness of the disease.

4.2 A less serious infection caused by *Legionella* bacteria is an illness known as Pontiac fever. The symptoms of Pontiac fever are similar to those of a mild to moderate influenza: headache, fatigue, fever, arthralgia (joint pain), myalgia (muscle pain) and, in a small proportion of cases, nausea, vomiting and coughing. The incubation period is short – less than one, up to a few days – and the illness generally self-resolves within three to seven days. No deaths have been attributed to Pontiac fever. Since this illness generally escapes detection, statistical information about its

occurrence is sparse. Pontiac fever is typically only detected in outbreak settings where as many as 90% of those exposed will develop symptoms of Pontiac fever.

4.3 Most healthy individuals are not at risk or become infected with *Legionella* bacteria after exposure. People at higher risk of getting sick are:

- a. Older people (usually 50 years of age or older)
- b. Current or former smokers
- c. Those with a chronic lung disease such as chronic obstructive pulmonary disease (COPD) or emphysema
- d. Those with a weak immune system from diseases like cancer, diabetes, or kidney failure
- e. People who take drugs that suppress or weaken the immune system, such as high dose corticosteroids, or those that undergo transplant operations or chemotherapy.

5. MICROBIOLOGY

5.1 *Legionella* is the name given to a genus of rod-shaped bacteria, often found in low concentration in natural waters. There are at least 60 different species named but one species, *Legionella pneumophila*, causes more than 90% of disease in the US.

5.2 *Legionella* are typically present in small numbers in most public drinking water distribution systems. Portions of these systems can provide a favorable environment for growth of *Legionella*. Transient upset conditions, such as water main breaks or heavy rains, may cause a change in water quality—either locally affecting individual buildings or municipal water supplies. This change may increase the bacteria in the water system, including *Legionella* bacteria. The potable/domestic water distribution systems of large buildings, including hospitals and hotels, are considered a primary source of *Legionella*

and disease as supported by data and expert sources, such as the CDC and peer-reviewed research studies.

5.3 Cooling towers, showers, washstands, sinks, air scrubbers and air washers / handlers can also provide a favorable growth environment and possible means of transmission of *Legionella* bacteria due to the creation of small droplets that can be inhaled. It should be noted that not everything emitted in the air leaving a cooling tower is drift that can contain *Legionella*.

NOTE: There is a common misconception what is seen as visible plume leaving cooling towers is all tower water drift and, thus, capable of containing and transmitting *Legionella*. However, just a very small portion (0.005% or less) of cooling tower circulating water is actually carried out of the tower as drift. This is equivalent to approximately 30 parts per million of the total air mass flow leaving a tower. The rest of the visible plume is (pure) water vapor from condensation of the cooling tower water evaporated by the normal operation of the cooling tower. When saturated air (100% humidity) contacts colder outside air, droplets of water condense, as in clouds in the sky, and are seen as a plume from the cooling tower. These water droplets do not contain *Legionella* as they are pure water condensate. During summer weather conditions, there is often no condensate formed and thus no visible plume. Drift is the very small portion of (actual) circulating cooling tower water that's been aerosolized into small water droplets and passes through or escapes the cooling tower drift eliminator structures (system), that ends up leaving the tower as drift and capable of containing and transmitting *Legionella*.

6. ECOLOGY

The ecology of *Legionella* in water systems is not fully understood; however, the following conditions have been found to affect its growth rate:

6.1 Sediment, corrosion deposits, mineral scale and organic materials can harbor the bacterium and promote growth.

6.2 The formation of a biofilm within a water system is thought to play an important role in harboring and providing favorable conditions in which *Legionella* can grow. A biofilm is a layer of microorganisms contained in a matrix that may form a thin layer of slime on surfaces in contact with water. *Legionella* grows within biofilms and within protozoa which shield *Legionella* from biocides that would otherwise kill or inhibit *Legionella* when the bacteria is freely suspended in water.

6.3 Water temperature in the range of 77°F (25°C) to 113°F (45°C) favor growth, though some *Legionella* strains may grow slowly outside this temperature range. The optimum temperature range for growth is approximately 97°F (35°C) to 104°F (40°C). *Legionella* will not proliferate below 68°F (20°C) or above 140°F (60°C). However, *Legionella* may remain viable and dormant in cool water and subsequently multiply when temperatures reach a favorable level and when growth and reproduction are not otherwise inhibited.

6.4 *Legionella* have been shown to colonize water systems that have areas of stagnation, e.g., water heaters, tanks, reservoirs, and basins. Fittings, piping, and various gasket materials used in these systems can also be colonized. Stagnant conditions may promote growth of *Legionella* and can provide areas of 'safe harbor' via biofilm, both of which can also make control difficult.

6.5 Commonly encountered microorganisms (such as algae, amoebae and other bacteria) in untreated or ineffectively treated water have been shown to promote *Legionella* growth. Many different types of protozoa serve as hosts for *Legionella*, which can enable rapid proliferation of *Legionella*. Certain bacteria have been shown to help *Legionella* to grow by providing growth promoting nutrients (Wadowsky, Stout).

7. PRACTICES & RECOMMENDATIONS FOR REDUCING RISKS ASSOCIATED WITH *LEGIONELLA*

7.1 Objectives

Cooling towers and evaporative condensers can provide favorable conditions for the growth and amplification of many microorganisms, including *Legionella*. Warm water temperatures, along with the presence of corrosion by-products, other deposits and sediment debris, further promote biofilm and can provide *Legionella* an environment for growth and amplification. Water chemistry and system maintenance should be well controlled in these systems. The objectives of any prudent water treatment program are to reduce corrosion, scale, deposits, and microbiological fouling in order to maintain efficient system operation, minimize energy consumption, and help to ensure safe operation. These same practices will also significantly contribute to the control of *Legionella* growth and amplification. A microbial control program, as discussed below, is just part of an effective water management program to control legionellosis.

Note that all water subsystems within a facility should be included in any effective legionellosis control system and water management plan and not just a focus on a single subsystem or piece of equipment. Therefore, a water management program for the control of legionellosis in a facility consists of other essential elements not discussed in this guideline. (see ASHRAE Standard 188 for reference).

Populations of bacteria will exist in any open loop cooling system and *Legionella* bacteria may be present. The goal of the microbial control program is not to sterilize the system, but rather to control amplification and prevent biofouling. The choice of microbial control

program is influenced by system variables such as makeup water quality, system design, operating parameters and potential for contamination. There are a wide variety of biocides and microbiological treatment products available. Reference Appendix E for guidance on the use of halogens for both continuous and intermittent uses for biological control in open loop systems. Halogens, such as chlorine and bromine, can be very effective in microbial control programs.

Ultimately, the efficacy of a microbial control program is affected by a variety of factors from product selection and application to system cleanliness and maintenance. Therefore, a performance-based method is recommended for confirming that the microbial control program employed meets stated objectives. Guidance on setting appropriate performance targets in measurable terms which can be validated is provided in section 7.3, Performance Monitoring.

7.2 Risk Assessment

As previously stated, this Guideline recommends a performance-based method for confirming that the microbial control program employed meets specific measurable objectives. The frequency of performance monitoring and the performance objectives are influenced by site-specific risk factors. These factors affect the potential for a given system to be associated with Legionnaire's disease. For instance, consider the dissemination and exposure to aerosols from the cooling water system – both where they are most likely to occur and the vulnerability of the population, given that the risks of Legionnaire's disease is highly correlated with exposure to susceptible population. Figure 1 is an illustration of a Legionnaires' disease risk profile.

Another opportunity for risk arises from non-routine operation such as during shut down and start up. Example guidelines for shut down and startup of evaporative heat rejection systems are provided in Appendices A-1 and A-2, for smaller and large systems, respectively. A water management plan, the integral component to be compliant with ASHRAE Standard 188, should include such protocols to reduce risk of exposure to *Legionella* bacteria during shut down and start up situations.

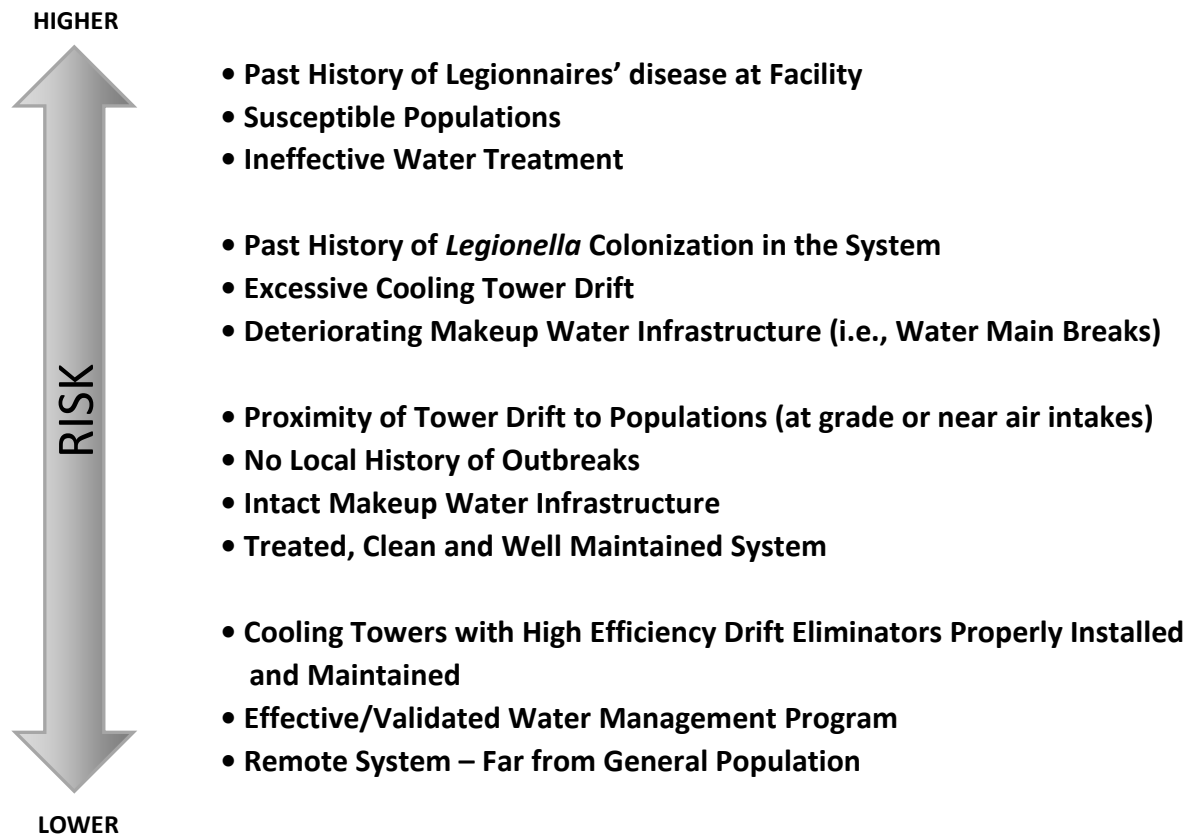


Figure 1. Legionnaires' Disease Risk Profile for Cooling Towers

7.3 Performance Monitoring

Performance monitoring is an essential part of the risk management process. Such monitoring will provide valuable information regarding the effectiveness of the current control program and more specifically, the efficacy of the applied microbial treatment regimen.

Performance monitoring is not restricted to just biological control parameters. All aspects within a control program should be monitored to ensure they remain effective and the risk is managed. This should include any mechanical, operational and/or chemical aspects of the overall program. Any monitoring to determine scale or corrosion will provide additional information.

Results and observations from monitoring performance will allow for remedial action to

be taken when required or will verify that control is being maintained. Documentation of monitoring results is verification that the intended monitoring activities and any necessary follow-up activities were completed. Guidance on roles, responsibilities and record-keeping is provided in section 8.

Commonly used monitoring techniques are described in the paragraphs 7.3.1 to 7.3.4.

7.3.1 Visual Inspection

It is important to maintain the cleanliness of both the system and the water in it and visual inspection of accessible areas will provide a qualitative audit of the current system cleanliness and potential for fouling. Safe access is required to all areas to be inspected

and when undertaking such inspections additional care should be applied when working at heights or in confined spaces.

Visual inspection of sections of the system may only be possible at predetermined intervals, for example, at system shutdown or when cleaning operations are undertaken.

Visual inspection can provide evidence of the presence of biofilm within the system, as well as inorganic and/or non-microbial foulants (for example, airborne or process contamination).

During the visual inspection certain mechanical failures may be identified. Particular attention should be paid to any visible damage to the drift eliminators, which may lead to emission of excessive drift or water spray. Refer to Appendix C for more details.

Methods: If access allows, the following areas should be inspected for fouling:

- Cooling tower basin and any other areas where water storage may occur
- The cooling water distribution system
- The cooling tower pack/ fill
- Distribution areas and/or nozzles
- Drift eliminators
- System components such as heat exchangers, filters, screens, strainers, and provisions for flushing low-flow/no-flow areas such as equalizer lines

Frequency: The frequency of visual inspections varies with the type of process, cooling tower size, location, and seasonality of operation. Other influences may also exist. These factors should be considered during the assessment of possible risk and the formulation of the inspection frequency.

The frequency of routine visual inspections should be at a minimum of once per month or per the manufacturer's recommendation, whichever is shorter. Events, such as system upsets, maintenance and prolonged idle periods

of operation may require more frequent inspection.

Performance Objectives: With respect to visual inspection, the following criteria indicate good practice:

- General system cleanliness: the system should be clean of organic fouling, dirt, or debris.
- The recirculating water should be clean and without excessive visible organic fouling.
- Stagnant areas in the cooling tower water system should be eliminated or regularly flushed.
- No excessive loss of drift from the tower should be evident. The drift eliminator section of the cooling tower should also be inspected for biological deposits, for significant damage and to ensure correct orientation and installation.
- Cooling tower fill should be free of excessive biomass, debris, and scale. If fouled, this may indicate that other parts of the system may be fouled, such as the water distribution within the cooling tower.
- Distribution areas should be free of biomass and debris and spray nozzles clean and free-flowing.
- Internal surfaces should be free of excessive corrosion byproducts and biomass.
- Cooling tower basin: inspect for evidence of biofouling, process contamination, and/or general cleanliness.
- No leaks should be evident, as this may reduce the concentration of water treatment chemicals.
- The water treatment equipment should be in a good condition and well maintained – all associated pumps and electrical connections should be functional.
- If present, sidestream filtration and basin sweeper piping and pump systems should be functional and well maintained.

7.3.2 Planktonic Monitoring Value

Trending of the concentrations of bacterial populations in the bulk water can be used to confirm that amplification is being controlled. Monitoring these bulk water planktonic populations is not a proxy for *Legionella* populations, but it is a valid monitoring tool for the continued and intended operation of the microbial control program. Increasing trends are indicative of a need to address all aspects of the microbial control program to assess the causes.

Methods: Dip slides, or other culturing techniques to quantify total aerobic heterotrophic bacteria populations in the bulk water, and ATP-based biomonitoring are commonly used microbial fouling monitoring methods. Results from culture techniques are reported as colony forming units of total aerobic heterotrophic bacteria per milliliter (cfu/ml). However, *Legionella* bacteria are not detected by these plate count media methods. ATP is measured as RLU (Relative Light Units). The acceptable level (as RLU) is a function of the specific monitoring equipment used. Correlation of ATP measurement to culture-based cfu/ml is system and method dependent. It is important to pick one monitoring method for consistent use. Periodically switching between monitoring methods may increase the probability of misinterpreting results due to differences in detection levels or reported units. In cases where the microbial control agent is applied intermittently, microbiological sampling should be completed just prior to an application of the microbial control agent and collected away from the point of injection. Systems where the microbial control program is applied continuously but supplemented periodically with an alternate biocide (oxidizer or non-

oxidizer) or dispersant (cleaner) treatment, should sample prior to the addition of the supplemental additive. However, these tests will not tell you whether *Legionella* is controlled.

Frequency: Site specific factors such as recirculating water quality and history of system cleanliness influence monitoring frequency. A typical minimum frequency is once per month. More frequent testing may be necessary if the system is subject to external factors such as process contamination, make-up water quality changes, or other issues and/or has a history of inadequate microbial control.

Performance Objectives: A heterotrophic count of 10,000 cfu/ml or less is a generally accepted operating range for most open cooling systems. This limit indicates that the microbial control program is providing a reasonable degree of system cleanliness, but it does not provide any guarantee that the system will be free of *Legionella* bacteria.

Very large systems such as power generators, may have difficulty attaining the accepted operating range before intervention. Many factors influence the level of organisms detected in the water including the nutrient level in culture media and incubation time and temperature.

Since the concentration of organisms depends on the specific method used, it is important to consistently test for microorganisms in the same way including the same culture media, incubation time, and as much as possible the same incubation temperature. Additionally, a control sample should always be run when using an ATP-based test method. The enzyme in the ATP test methods can degrade over time making a correlation to the control sample important.

As long as the testing method remains constant, an increase of microorganism counts greater than two logs – i.e., 10^3 cfu/ml to 10^5 cfu/ml (1,000 cfu/ml to 100,000 cfu/ml) should trigger a reevaluation of the microbial control program.

The following (Table 1.) is an example response plan when monitoring a cooling tower water system for total bacteria (Total Aerobic Heterotrophic Plate Count).

**Table 1. Example Response Plan for Total Aerobic Bacteria
(Heterotrophic Plate Count / HPC)**

Total Aerobic Bacteria Test Result (cfu/ml)	Response to Total Aerobic Bacteria Count
<10,000 cfu/ml	1. Maintain monitoring schedule and water treatment program.
$\geq 10,000$ cfu/ml, but <100,000 cfu/ml	2. Review the water treatment program, make appropriate or corrective actions to reduce the total aerobic bacteria (count) to <10,000 cfu/ml. Document review/efforts made and follow response action 1.
$\geq 100,000$ cfu/ml	3. Review the water treatment program, make appropriate or corrective actions to reduce the total aerobic bacteria (count) to <10,000 cfu/ml. Document review/efforts made and follow response action 1. If test results are a second <u>consecutive</u> scheduled monitoring report of $\geq 100,000$ cfu/ml, follow Response 4. 4. Perform an on-line (or off-line) disinfection; examples are provided in Appendix A-3 and A-4. After disinfection, retest the water within 3-7 days after the system has returned to normal operating conditions and follow response plan 5. Per the total bacterial retest results, proceed as follows: a. If retest is <10,000 cfu/ml, follow Response 1. b. If retest is >10,000 cfu/ml but <100,000 cfu/ml, follow Response 2. c. If retest is $\geq 100,000$ cfu/ml, repeat Response 4.