



Legionella: A Growing Problem

Increasing infection rates create need for efficient testing methods

BY DIANA HULBOY

Just 40 years ago, *Legionella* bacteria were identified as the cause of Legionnaires' disease, a deadly pneumonia, and Pontiac fever; since that time, infection rates have increased. New, unexpected modes of transmission are arising, and recent events have implicated source water supplies and drinking water infrastructure in addition to premise plumbing in the growth and spread of *Legionella*. There is a need for rapid, onsite, sensitive, quantitative *Legionella* monitoring.

Current Understanding of *Legionella*

According to the Centers for Disease Control and Prevention (CDC), *Legionella* infections are underreported, but are responsible for 8,000 to 18,000 hospitalizations each year in the U.S. alone, with a 5% to 30% mortality rate and a 219% increase in reported cases from 2000 to 2009.

Because *Legionella* bacteria prefer warmth, water and iron, they typically occur in hot water systems. However, *Legionella* can survive and grow in a range of temperatures and conditions, and because infection occurs via inhalation, any

process that creates aerosols—beach showers, humidifiers, spas, pools, car washes—can be at risk for spreading *Legionella*. Infection also is possible via accidental aspiration while drinking water, swimming or gardening. Recently, evidence of person-to-person transmission was reported in the *New England Journal of Medicine*.

Because the elderly, ill, smokers and immunocompromised are especially vulnerable to *Legionella* infection, hospitals and retirement homes are particularly at risk, but a significant number of hotels and cruise ships have experienced outbreaks as well. Each week there are new reports of *Legionella* contamination, and several high-profile outbreaks have occurred recently, including in Flint, Mich., with nine deaths in 2014 and 2015, and a hotel in the South Bronx in New York City last summer resulting in 12 deaths, which prompted a New York state regulation on *Legionella* monitoring in cooling towers.

More unusual causes of *Legionella* transmission are being reported as well. In 2010, a Wisconsin hospital's outbreak was linked to a decorative fountain. Elsewhere, grocery store produce misters

Cooling towers (evaporative condensers, usually on building rooftops) are a common source of *Legionella* contamination because their wet conditions provide a good environment for growth of the bacteria.

have been the culprits. Particularly surprising is the spread of *Legionella* by trucks used for street paving and cleaning—outbreaks in Alcoi and Ripollet, Spain, in 2009 and 2014, respectively, resulted in 59 cases and 11 deaths. In both cases, identifying the vehicles responsible and taking them out of service put an end to the outbreak.

Implications for Source & Drinking Water

Although industry-related sources of *Legionella*, such as cooling towers, tanks and hot water systems, are well documented, CDC reports that drinking water-associated outbreaks involving bathroom showers or hospital nebulizers, for example, also have been on the rise. For this reason, the U.S. Environmental Protection Agency (EPA) is considering including *Legionella* in its Fourth Unregulated Contaminant Monitoring Rule list of drinking water contaminants that require monitoring.

The increase in drinking water-related cases may be due to changes in drinking water sources.



The Legipid method utilizes a color change to rapidly and accurately detect and measure the presence of *Legionella* in samples.



Culturing of suspected samples on bacterial culture plates, the primary method of *Legionella* identification, is accurate and quantitative, but can take up to 14 days.

Legionella occurs naturally in water and soil, but changing environmental factors may be creating more favorable conditions for growth. Increased nutrients from pollution, including iron, fertilizer and organic waste, become concentrated as freshwater supplies dwindle in drought-stricken areas. Additionally, increasing temperatures encourage *Legionella* growth, especially in areas with higher humidity.

Algae blooms, which are on the rise around the world, deplete oxygen in lakes and rivers. Robert Wadowsky's group reported that *Legionella* can survive low dissolved oxygen levels, potentially giving it a growth advantage over other bacteria (Applied and Environmental Microbiology, 1985).

Finally, when salt is applied to icy roads in the winter, runoff feeds it into surface water and groundwater, making them more corrosive over time. According to Marc Edwards' research group at Virginia Tech, the high corrosiveness of Flint River water was the cause of the lead contamination of Flint's water supply, and also may have led to its *Legionella* outbreak; prior to the two years in which Flint River water was used, the infection rate was much lower. The researchers explained that because the new water supply was not treated with a corrosion inhibitor, the water removed the protective phosphate layer from the inside surfaces of pipe, corroding the metal and causing release of lead—and iron—into the drinking water. Iron, in addition to being a nutrient required for *Legionella* growth, inactivates chlorine disinfectant, to which *Legionella* is somewhat resistant, as demonstrated by EPA's monitoring of

Flint's drinking water. These conditions provide an ideal growth environment for *Legionella*.

Flint's drinking water system experienced significant changes in water pressure when it switched water sources, as well as water main breaks after the aging infrastructure was further compromised by the new source water. These events likely disrupted biofilm, limescale and sediment within the pipe, which, according to EPA's 1999 *Legionella* Human Health Criteria document, can harbor *Legionella* bacteria. This may have contributed to the spread of *Legionella* throughout the Flint drinking water system.

Thus, if widespread changes are causing an escalation in incidence and levels of the bacteria in the environment, this would enhance "seeding" of *Legionella* into infrastructure and premise plumbing, and could explain the increase in drinking water-associated, as well as industry-associated, outbreaks.

New Detection Technology

Given the increasing incidence of *Legionella*, it is important to be able to quantitatively detect the bacteria rapidly, accurately, sensitively, and on site, enabling timely application of disinfectant and substantially reducing the risk of infection. None of the traditional detection methods meets all of these criteria.

Culturing, the primary method, is mostly accurate and quantitative, but slow (seven to 14 days), and, for several reasons, subject to false negatives. Polymerase chain reaction (PCR) is faster, but not rapid (eight to 24 hours) nor quantitative

(in terms of colony-forming units), and is subject to both false positive and negative results. Both methods are expensive, cannot be performed on site and require scientific expertise. Strip tests are rapid, but not quantitative, and only detect *Legionella pneumophila*, the most common, but not the only deadly, species of *Legionella*.

A new method called Legipid uses immunomagnetic separation capture enzyme immunoassay (IMS-CEIA). The AOAC-certified method is fast, with the ability to perform up to 40 tests one hour after filtering. It is as sensitive as culturing and PCR and is selective for 90% of *Legionella* species, including *Legionella pneumophila*. It offers visual (semi-quantitative) or optical (quantitative) colorimetric detection and detects only live *Legionella*, including viable but not culturable bacteria. Few false negatives or positives are produced.

The continued increase in *Legionella* incidence heightens the need for a reliable and rapid test method. The new IMS-CEIA test can be used by facility employees, enabling prompt remediation while a subset of samples are sent for confirmation by culture testing. **CW**

Diana Hulboy, Ph.D., is strategic leader, operations and business development, for Abraxis LLC. Hulboy can be reached at dhulboy@abraxiskits.com or 215.357.3911. L. Kamp and M. Lázaro provided editorial review of this article.