Regulating Legionella: Choosing an Analytical Method
By Christopher Goulah

During the summer of 2015, a series of fatal legionellosis outbreaks forced the New York City (NYC) Department of Health and Mental Hygiene (DOHMH) to take emergency action to contain the rapid spread of the infection. After determining that the source of the outbreak was a centrally located cooling tower, emergency ordinances were put in place that mandated the disinfection of all cooling towers citywide. The city passed permanent regulations to maintain a set schedule of maintenance and biological monitoring of all cooling towers within the NYC limits. Requirements included daily water quality measurements, weekly biological process control indicators, and quarterly Legionella testing. NYC DOHMH quickly followed suit and published “Protection Against Legionella,” which established similar regulations statewide to cover both cooling towers and healthcare facilities. The state regulations require owners of cooling towers and healthcare facilities to have a water management plan in place and to monitor their water systems for biological content at regular intervals.

NY Sampling Requirements

Sampling for Legionella in cooling towers is required every 90 days from one or more of the following locations: tower sump, tower makeup, heat exchanger inlet/outlet, or the tower pack. It's recommended that healthcare facilities sample 10 percent of selected outlets—both hot and cold—semi-annually. In both cases, the samples must be collected in sterile vessels containing sufficient sodium thiosulfate to neutralize any residual chlorine present. This is essential to prevent continued disinfection of the sample during transport, which could result in a false negative result. Similarly, the sample must be transported on ice to prevent continued growth of any bacteria present that could overwhelm or inhibit the slow-growing Legionella. It is recommended that samples arrive at the environmental testing laboratory within 48 hours of collection.

New York State (NYS) requires all Legionella samples to be analyzed at a laboratory certified by the Environmental Laboratory Approval Program (ELAP). In order to obtain certification from NYS, commercial Legionella labs are required to isolate and enumerate Legionella bacteria according to ISO method 11731:1998 for potable and non-potable samples. Previously, most commercial labs both in NYS and nationwide followed the method prescribed by the Centers for Disease Control and Prevention (CDC) and utilized CDC’s Environmental Legionella Isolation Techniques Evaluation (CDC-ELITE) proficiency testing program. While the two methods—CDC and ISO—operate on the same fundamental
principles, there are methodological differences that can result in variances in the detection of *Legionella* from identical environmental samples.

**Methodological Differences**

One of the more critical differences between the two methods is the way in which non-potable water samples are initially processed. The CDC method directs all non-potable samples to be directly plated onto selective buffered charcoal yeast extract (BCYE) media, both with and without various antibiotics and in the presence and absence of the amino acid cysteine, which is a strict nutritional requirement of *Legionella*. Direct plating of water samples provides a limit of detection (LOD) of 10 CFU/mL based on the common practice of 0.1 mL being plated. Current NYC regulations set the action level for the presence of *Legionella* in cooling towers at 10 CFU/mL; at that level, immediate disinfection, review of the treatment program, and retesting of the waters within three to seven days is required. Therefore, any positive sample using the CDC method would require immediate action with no warning that *Legionella* may be starting to proliferate in the system.

In contrast, the ISO method directs all samples (potable and non-potable) to be concentrated prior to plating. Typically, a 250 mL water sample submitted for analysis that is concentrated and subsequently resuspended in 5 mL of isotonic buffer prior to plating can lower the LOD to 0.2 CFU/mL. This lowered LOD provides a buffer zone to the maintenance engineers so that when *Legionella* are detected, there is an opportunity to readjust the disinfection protocol to remain compliant without triggering a regulatory action.

Another difference between these two methods is the secondary processing of the sample prior to plating. The CDC method directs samples with high background to be reprocessed using acid treatment while the ISO method mandates that all samples be independently acid treated and heat treated. The heat treatment has been validated by multiple studies to be more effective at decreasing the levels of background flora that normally flourish on the highly selective BCYE media even in the presence of multiple antibiotics. This is particularly useful with regard to reducing bacterial species that are inhibitory to *Legionella* growth; for example, the presence of *Pseudomonas aeruginosa* can result in false negative results.

The last methodological difference does not relate to the efficacy of the process, but rather to the overall efficiency and cost to perform the test. The CDC method utilizes four variations of the BCYE agar during the initial plating of the sample: BCYE; BCYE with Polymyxin B, Vancomycin and Cycloheximide (PVC); PVC with glycine (GVPC); and GVPC without cysteine (GVPC-). In addition, the PVC and GVPC agar are plated in duplicate, resulting in a total of six plates. If acid treatment is required to reduce background flora, as is usually the case for non-potable samples, this is doubled to 12 plates. The ISO method performs the secondary acid and heat treatments up front and plates each treatment on a single GVPC plate. The heat treatment is also plated on the GVPC- plate as a negative control, bringing the total number of plates utilized during the ISO method to four. The decrease in the number of plates combined with the amount of time saved by virtually eliminating the need to reprocess the samples results in a highly streamlined method.

**Which Method?**

As additional states follow New York’s lead and start to regulate testing for *Legionella*, the question of which method to utilize will come up more frequently. Based on the procedural differences between the CDC and ISO methods, the ISO method a more appropriate choice for regulators, engineers, and environmental laboratories.

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