



Scientific Brief

Achieve Greater Accuracy and Recovery in *Legionella* Water Testing

Introduction

The monitoring of *Legionella* is critical to ensure public health. There are at least 59 different *Legionella* species with some of these strains infecting humans. The bacteria is the leading cause of waterborne disease outbreaks in the United States. The common sites of *Legionella* transmission are heating and ventilation systems, potable water systems used for human showering, cooling towers, and decorative fountains. The water in these systems must be tested on a regular basis as part of a facility's comprehensive water quality management program.

Testing water for the presence of *Legionella* bacteria is reliably accomplished by culture in an accredited microbiology laboratory using well-characterized and validated methods. The most pathogenic (disease-causing) strain of *Legionella* bacteria is the *L. pneumophila* group. Another pathogenic strain is *L. anisa*.

With the COVID pandemic closing many institutions and office buildings, concern has mounted over stagnant water in these structures' HVAC systems, plumbing, public pools and cooling towers. As the pandemic abates and these facilities come back into use, the result could drive an increase in *Legionella* testing to detect and prevent outbreaks.

Government regulations require a testing methodology that filters water samples through a permeable 0.45 micron (or smaller) membrane. Bacteria is concentrated on the membrane, which is placed on media to foster its growth. After about 7 days of incubation, the bacterial colonies are counted to determine the *Legionella* bacteria's presence and density.

The membrane filter is a key component in the accuracy and recovery of the test. However, it has been suspected that different membrane materials offer varying levels of recovery performance.

This scientific brief reviews studies (found in the References section) that were conducted to review the performance of black polyethersulfone (PES) membrane and several other commonly used membrane materials per the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*." The membrane materials were tested to determine their recovery performance for *L. pneumophila* and *L. anisa*.

Black PES Membrane Achieves Higher *L. pneumophila* Recovery than Other Filters Tested

In this study, a black PES membrane filter, such as the one found in Pall Laboratory's Metrice^l® black membrane filter, was quantitatively compared to two black nitrocellulose membrane (NCM) filters available on the market. The study was performed by a third-party laboratory and consisted of three replicates from three lots of Pall's (Supplier 1) black PES membrane filter, two lots of a black NCM membrane filter from a second supplier, and one lot of black NCM membrane filter from a third supplier.

Results

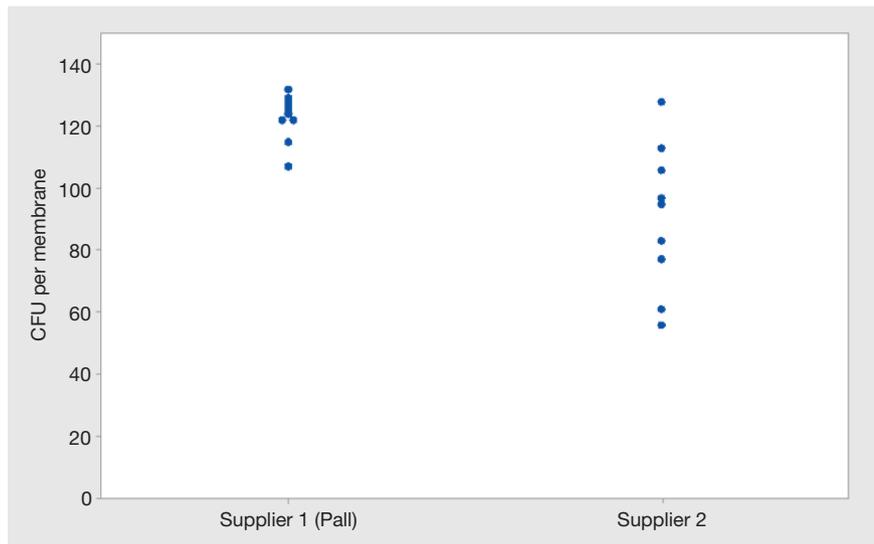
Colonies on all membrane filters were uniformly distributed and easily counted. No leaching of color was observed. The average CFU/membrane counts of *Legionella pneumophila* on Supplier 1's black PES membranes and the black NCM membranes from Supplier 2 and 3 are provided on Table 1. Membrane types, CFUs, and the averages also are listed in Table 1. Individual value plots between Supplier 1's black PES membranes and Supplier 2's black NCM membranes are graphed on Figure 1.

Table 1*Results of Legionella pneumophila Recovery on Three Different Black Membranes*

Membrane Type	Lot	Colony Forming Units <i>Legionella pneumophila</i>			Mean
Supplier 1 Black PES (Pall)	Lot 1	125	127	132	128
	Lot 2	124	129	107	120
	Lot 3	115	122	122	120

Membrane Type	Lot	Colony Forming Units <i>Legionella pneumophila</i>			Mean
Supplier 2 Black NCM	Lot 1	106	97	77	93
	Lot 2	94	56	83	78
	Lot 3	128	61	113	101

Membrane Type	Lot	Colony Forming Units <i>Legionella pneumophila</i>			Mean
Supplier 3 Black NCM	Lot 1	72	54	59	62

Figure 1*Individual Value Plot of Supplier 1 and Supplier 2*

The morphology of colonies on all black membrane filters at 4 days was typical of *Legionella pneumophila* (Figure 2). The light gray colonies had slight ground glass appearance and iridescence. By 7 days and 10 days, most colonies on the black PES membranes appeared light tan rather than light gray in color with some colonies light to darker brown in color. Many of the smaller colonies on Supplier 2's black NCM membranes still appeared light gray in color with larger colonies becoming tan to light or darker brown. On all black membranes, the larger colonies became more transparent with less ground glass appearance and iridescence. On Supplier 3's black NCM membranes, the colony morphology and changes over the 10-day incubation were similar to black PES membranes. However, with fewer colonies present, the larger, well-isolated ones were flattening by 10 days.

Notably, the black gridlines on Supplier 2's black NCM membrane were indented in the surface of the filter (Figure 3). This caused colonies growing on top and next to the gridlines to form irregular edges and appear misshapen. The gridlines on Supplier 1's black PES membrane and Supplier 3's black NCM membrane were flat, so colonies were round and convex, which is typical (Figure 2).

The black PES membrane filter demonstrated higher recovery versus the black NCM membranes, demonstrating equivalency for use in meeting International Standard ISO 11731.

Figure 2

Supplier 1 (Pall) Black PES membrane under microscope Day 7

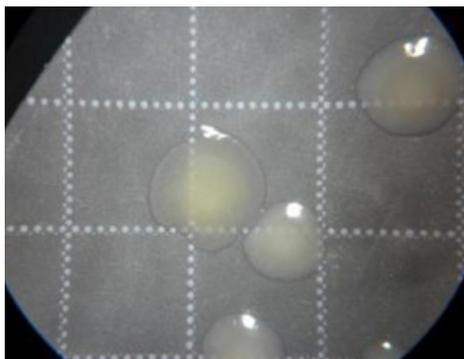
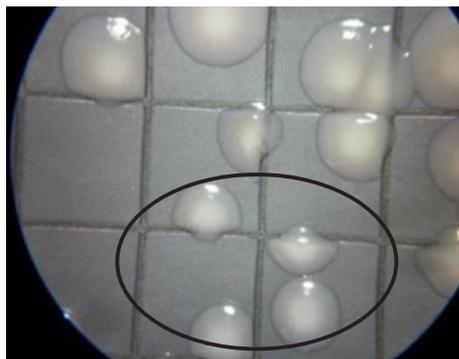


Figure 3

Supplier 2 Black NCM under microscope Day 7



Black PES Membrane Outperforms NCM Membrane in Capture of *Legionella anisa*

As we have noted in the introduction, the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*," describes intra-laboratory and inter-laboratory data collected to evaluate performance characteristics of the procedure. In June 2016, an inter-laboratory study was carried out by ten laboratories to evaluate the method's performance characteristics using membrane filtration with direct placing of the membrane filter on the culture media.

Problems were observed with the *L. anisa* samples. Seven out of the ten laboratories were unable to grow *L. anisa* on the nitrocellulose or mixed cellulose ester membrane used for this method. The three laboratories that were able to grow *L. anisa* reported a maximum concentration of 900 cfu/L, whereas the concentration by direct plating method was found to be 36,000 cfu/L.

It was therefore of interest to determine if recovery could be improved through the use of a PES membrane, rather than cellulose nitrate or mixed cellulose ester. The study was performed by a third-party laboratory and consisted of three replicates from three lots of Pall's (supplier 1) black PES membrane filter and Supplier 2's black NCM membrane filter. Only one lot from Supplier 3's black NCM membrane filter was available for testing in triplicate. Therefore, this was included as additional information for reference.

Results

Similar to the results reported in International Standard ISO 11731, "Water quality – Enumeration of *Legionella*," no *L. anisa* was recovered from the NCM. In contrast, average recovery of *L. anisa* from the three lots of black PES membranes ranged from 98% to 119% of the calculated inoculum.

The *L. anisa* grew on all of Supplier 1's black PES membrane filters. The CFU/membrane filter averages for the three lots were 39, 32, and 34 (Table 1). The theoretical inoculum for each membrane was 33 cfu (the colony count of the 10⁻⁵ suspension was 330 CFU/mL, diluted 1:1000). *L. anisa* did not grow on any of Supplier 2's or Supplier 3's black NCM membranes.

Table 2

Membrane Type	Lot	Colony Forming Units <i>Legionella anisa</i>					
		Plate 1	Plate 2	Plate 3	Average	Std Dev	Theoretical Recovery (%)
Supplier 1's black PES (Pall)	Lot 1	33	40	45	39	9	119
	Lot 2	34	30	33	32	5	98
	Lot 3	43	28	32	34	11	104

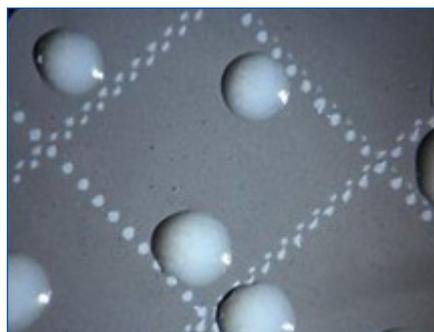
L. anisa did not grow on any of Supplier 2's or Supplier 3's Black NCM membranes.

Colonies of *L. anisa* on the PES membrane filters were uniformly distributed, easily counted, and no leaching of color was observed. Colony morphology at four days was typical: whitish with a slight ground glass appearance and iridescence (Figure 4). By seven days and ten days, colonies appeared more beige than white in color and the periphery of the older, larger colonies became slightly transparent. Under UV light, colonies fluoresced blue-white, weakly at four days but with greater intensity by seven days. At four days, *L. anisa* colonies on the PES membrane filters had typical color and morphology: whitish colonies with slight ground glass appearance and iridescence. Colony growth is not impeded by grid markings.

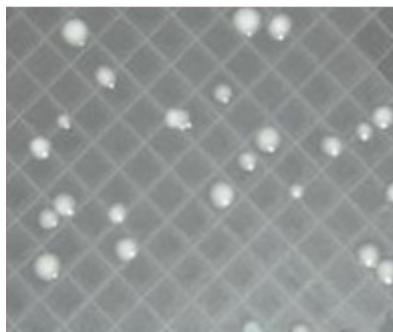
Figure 4

Colony morphology on Metricel black PES membrane

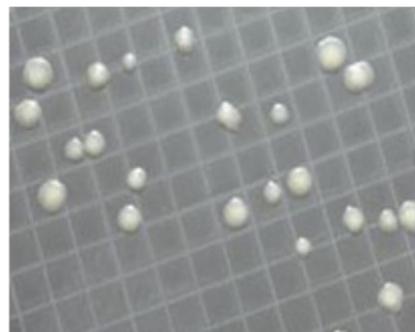
Colonies on Day 4



Colonies on Day 7



Colonies on Day 10



Materials and Methods

The pore size and membrane material selection used in these studies were chosen based on accepted membrane filter applications outlined by the international standard for *Legionella* testing in water and alternative membranes that are commercially available. All testing was done per the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*."

For additional testing details, please see the Application Notes in the References section.

Conclusion

Pall's (Supplier 1's) black PES membrane filters showed significantly better performance than Supplier 2's black NCM membrane filters in the recovery of the *L. pneumophila* serogroup 1 from a pure culture-spiked water sample. Both membranes recovered more *Legionella* than Supplier 3's black NCM membrane. In contrast, colonies growing on or next to the indented gridlines of Supplier 2's black NCM membranes were misshapen; i.e., not round as they were elsewhere on the membrane surface.

In the *L. anisa* study, the bacteria was able to grow without apparent inhibition from a pure culture inoculum on all three lots of Supplier 1's black PES membrane filter with average recoveries ranging from 98% to 119% of the calculated inoculum. The *L. anisa* colonies grown exhibited typical colony morphology with no distortion or inhibition from the grid lines. In contrast, no growth of *L. anisa* was observed on the NCM membrane filters. The latter observation mimics results obtained in the inter-laboratory studies carried out for the validation of the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*." These results confirm the conclusion offered in Annex H of the method that the choice of membrane can strongly influence the growth of *L. anisa* and suggest that a PES membrane should be considered for recovery of this organism.

Overall, these comparison studies support that the use of black PES membrane filters for recovery of *Legionella* bacteria in water samples provides more accurate results and greater recovery performance than the other materials tested.

Pall Laboratory offers a comprehensive line of filters for *Legionella* water testing workflows.

For more information or free samples, visit www.pall.com/legionella.

References

- *Evaluation of Pall Metrical Black PES membrane Filter for Legionella pneumophila by Direct Membrane Filtration Method*,
<https://laboratory.pall.com/content/dam/pall/laboratory/literature-library/gated/L-pneumophila.pdf>
- *Evaluation of Pall Metrical Black PES Membrane Filter for Legionella anisa by Direct Membrane Filtration Method*
<https://laboratory.pall.com/content/dam/pall/laboratory/literature-library/gated/L-anisa.pdf>



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