



Application Note

Evaluation of Pall Metrice[®] Black PES membrane Filter for *Legionella pneumophila* by Direct Membrane Filtration Method

Summary

Monitoring of legionellae is critical for public health reasons. There are at least 59 different *Legionella* species. In about half of those species, some strains infecting humans have been reported. This application study demonstrates the recovery performance of Pall's Metrice Black Polyethersulfone (PES) membrane filter of *Legionella pneumophila* per the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*".

Pall's Metrice Black PES membrane filter was quantitatively compared to two black nitrocellulose membranes (NCM) filters which are currently available on the market. The study was performed by a third party laboratory and consisted of three replicates from three lots for Pall's Metrice Black PES membrane filters and Competitor 1's Black NCM membrane filters. Only one lot from Competitor 2's Black NCM membrane filter was available for testing in triplicate therefore this was included as additional information for reference. Pall's Metrice Black PES membrane filters demonstrated higher recovery vs the black competitor NCM membranes, demonstrating equivalency for use in the International Standard ISO 11731.

Materials and Methods

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

The membrane filters used were Pall's Metrice Black PES membrane, and two competitor's black nitrocellulose membrane filters. All membrane filters used had a 0.45 μm pore size. The Competitor 1 Black NCM membrane filter required autoclaving prior to testing while the other membranes were provided sterile. All the testing was done aseptically.

Preparation of Inoculum

Legionella pneumophila, serogroup 1 (ATCC 33152, Lp1), was grown overnight on Buffered Charcoal Yeast Extract agar (BCYE). A primary suspension was prepared in sterile Deionized (DI) water to a turbidity that equaled a 0.5 McFarland standard as measured on a Vitek turbidity meter. At this turbidity, the Lp1 yielded a concentration of approximately 10^8 CFU/mL. The primary suspension was diluted out to 10^{-5} by transferring 1 mL serially in 9 mL of 1:40 Ringer's solution. To prepare the single inoculum, 3 mL of the 10^{-5} dilution were transferred to 3,000 mL of the 1:40 Ringer's solution in a large flask, stirred to mix, then split into 3 sterile bottles for easier handling. 100 mL of inoculum would theoretically inoculate each membrane ~100 CFU.

Filtration of Inoculum

All membranes were tested within 30 minutes of inoculum preparation. Membrane filters were aseptically transferred to separate sterile filtration units. Using a sterile, 100 mL graduated cylinder, 100 mL of the inoculum was transferred to the separate funnels and filtered. As a rinse, 20 mL 1:40 Ringer's solution was transferred to and filtered through each membrane. Each membrane was aseptically removed from the filtration unit and placed facing upwards on a BCYE plate. Plates were C for 10 days. Cultures were examined with colony counts and observations recorded at 4 days, 7 days and 10 days.

Results

Colonies on all membrane filters were uniformly distributed, easily counted, and no leaching of color was observed. The average CFU/membrane counts of Lp1 on Pall PES membranes, Competitor 1 Black NCM, and Competitor 2 Black NCM are given on Table 1. The final colony counts on the Competitor 2 membranes were lower with an average count of 62 CFU/membrane and used as additional information. Membrane types, CFUs and the averages are listed in Table 1. The Standard Deviation (SD) for each batch and reproducibility taking into account the three lots tested for Pall Black PES membranes and Competitor 1 Black NCM is shown on Table 2. Individual value plots between Pall and Competitor 1 Black NCM are graphed and seen on Figure 1.

Table 1

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

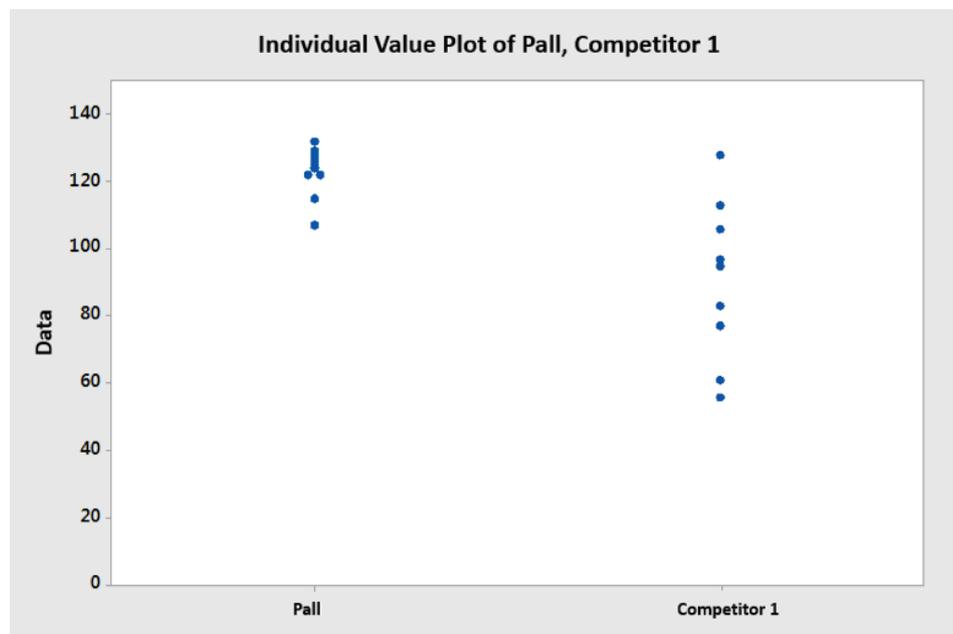
Membrane Type	Lot	Colony Forming Units <i>Legionella pneumophila</i>			Mean
Competitor 1 Black NCM	Lot 1	106	97	77	93
	Lot 2	94	56	83	78
	Lot 3	128	61	113	101

Membrane Type	Lot	Colony Forming Units <i>Legionella pneumophila</i>			Mean
Competitor 2 Black NCM	Lot 1	72	54	59	62

Table 2

	Lot	Pall Black PES		Competitor 1 Black NCM	
		Mean	SD	Mean	SD
Repeatability	1	128.0	3.61	93.3	14.84
	2	120.0	11.53	78.0	19.97
	3	119.7	4.04	100.7	35.16
Reproducibility	Mean (CFU/sample)	122.56	122.56	90.67	90.67
	SD	7.57	7.57	23.75	23.75

Figure 1



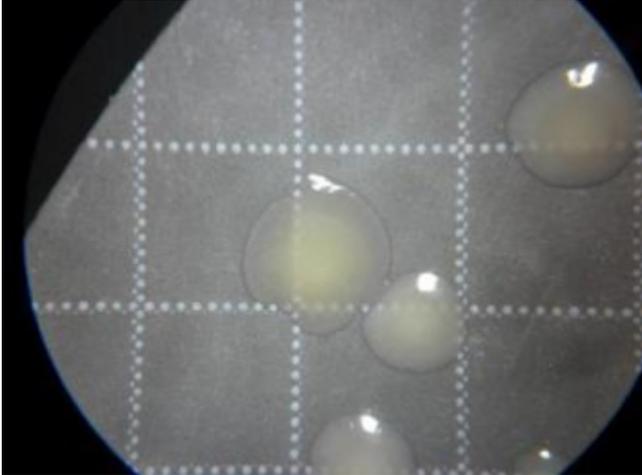
The morphology of colonies on all black membrane filters at 4 days was typical of Lp1. The light gray colonies had slight ground glass appearance and iridescence.

By 7 and 10 days, most colonies on the 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 the Competitor 1 Black NCM 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 and iridescence. On the Competitor 2 Black NCM, the morphology colonies and changes over the 10-day incubation were like the PES membranes; however, with fewer colonies present, the larger, well isolated ones were flattening by 10 days.

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

Figure 2

Pall Metricel Black PES Membrane under microscope Day 7



Pall Metricel Black PES Membrane Day 7

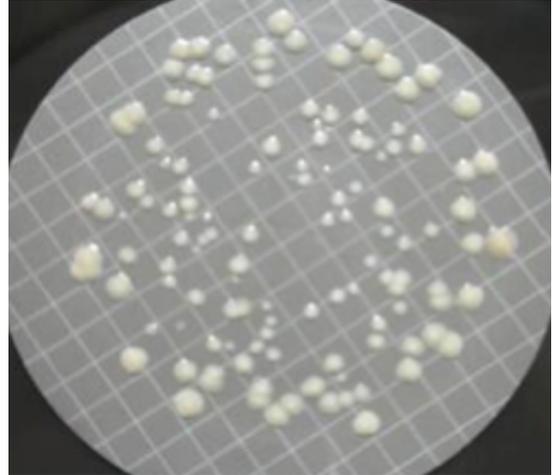
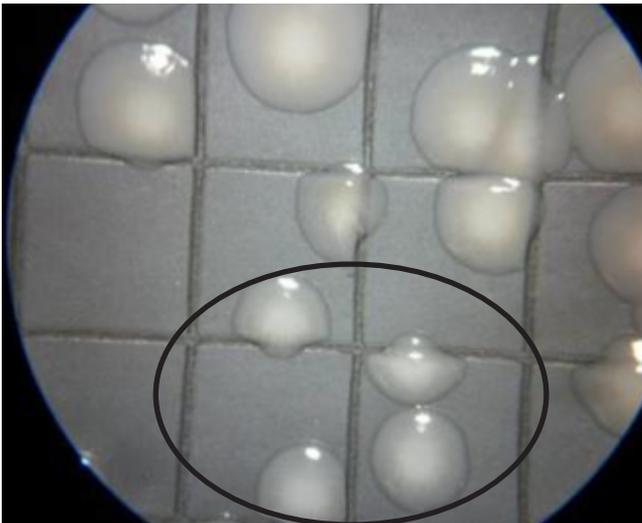
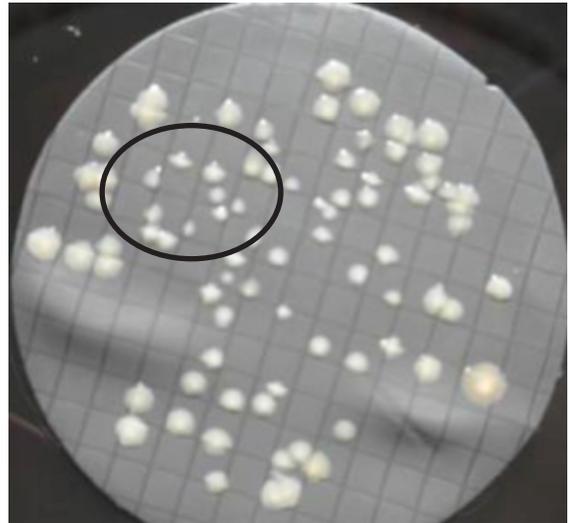


Figure 3

Competitor 1 under microscope Day 7



Competitor 1 Black NCM Day 7



Figures 2 and 3: Legionella colonies on a Pall Black PES membrane compared to Lp1 colonies in Competitor 1 Black NCM that was evaluated on day 7. On the PES membrane the Lp1 colonies had typical round shape; however, on the Competitor 1 Black NCM, the indented gridlines caused the Lp1 colonies to be irregularly shaped. The membranes appear white or gray in the photographs though they were both black on the surface of the BCYE.

Conclusion

In this study, the Pall MetriceL PES membrane filters showed significantly better performance than Competitor 1 black nitrocellulose membrane filters in the recovery of the *L. pneumophila* serogroup 1 from a pure culture spiked water sample performed by a third party laboratory. Both recovered more legionella than the Competitor 2 Black NCM membrane filter. Color of the colonies on PES and NCM black membrane filters were the same except for colony shape. In contrast, colonies growing on or next to the indented gridlines of Competitor 1 Black NMC membrane filters were misshapen; i.e., not round as they were elsewhere on the membrane surface. This comparison study supports that the use of Pall MetriceL Black PES membranes for recovery of Legionella in water samples provides more accurate results.



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