



Application Note

Evaluation of Pall Metrice[®] Black PES Membrane Filter for *Legionella anisa* 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, strains infecting humans have been reported. This application study demonstrates the recovery performance of Pall's Metrice Black Polyethersulfone (PES) membrane filter of *Legionella anisa* per the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*".

Annex H of the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*" (draft 4), describes intralaboratory and interlaboratory data collected to evaluate performance characteristics of the procedure. In June 2016, an interlaboratory study was carried out by 10 laboratories from 4 countries to evaluate the performance characteristics of the method using membrane filtration with direct placing of the membrane filter on the culture media. Two samples were used each spiked with a different *Legionella* species, *L. pneumophila* or *L. anisa*. For the *L. pneumophila* samples, a repeatability of 51.1% and a reproducibility of 65.9% was reported. However, problems were observed with the *L. anisa* samples. Seven out of the ten laboratories were unable to grow *L. anisa* on the cellulose nitrate 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. The authors concluded that "in this case the membrane filter strongly influenced the growth of *L. anisa* on the membrane filter."

It was therefore of interest to determine if recovery could be improved through use of a membrane of a different base material, polyethersulfone, rather than cellulose nitrate or mixed cellulose ester. Three lots of Pall Metrice Black polyethersulfone membrane filters (PES) were quantitatively compared to two other black nitrocellulose membranes (NCM) filters which are currently available on the market. This 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.

Similar to the results reported in Annex H of the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*" (draft 4), no *L. anisa* was recovered from the nitrocellulose membranes. In contrast, average recovery of *L. anisa* from the three lots of Pall's Metrice Black PES membranes ranged from 98 to 119% of the calculated inoculum. These results confirm that choice of membrane can strongly influence the growth of *L. anisa* and suggest that a polyethersulfone membrane should be considered for recovery of this organism.

Materials and Methods

The membranes and pore size 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 performed per the International Standard ISO 11731, “Water quality – Enumeration of *Legionella*”.

The membrane filters used were Pall’s Metrical 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 anisa (ATCC 35292), was grown overnight on Buffered Charcoal Yeast Extract agar (BCYE). A primary suspension was prepared in sterile DI water to a turbidity that equaled a 0.5 McFarland standard as measured on a Vitek turbidity meter. At this turbidity, the concentration of *L. anisa* was approximately 10⁸ CFU/mL. The primary suspension was diluted to 10⁻⁵ by transferring 1 mL serially through tubes of 9 mL 1:40 Ringer’s solution. To prepare the inoculum, 3 mL of the 10⁻⁵ dilution were transferred to 3,000 mL of 1:40 Ringer’s solution in a large flask, stirred to mix, then split into 3 sterile, 1 L bottles for easier handling. 100 mL of inoculum would theoretically inoculate each membrane filter with ~100 CFU.

Filtration of inoculum

All membrane filters 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 filter was aseptically removed from the filtration unit and placed facing upwards on a BCYE plate. Plates were incubated in humidified air at 36 ± 1 °C for 10 days. Cultures were examined at 4, 7 and 10 days to observe growth and count colonies.

Results

The *L. anisa* grew on all Pall PES membrane filters evaluated. The CFU/membrane filter averages for the three lots were 39, 32, and 34 (Table 1). The colony count of the 10⁻⁵ suspension was 330 CFU/mL, once diluted to 1:1000, the final inoculum would have theoretically inoculated each membrane with 33 CFU.

Table 1

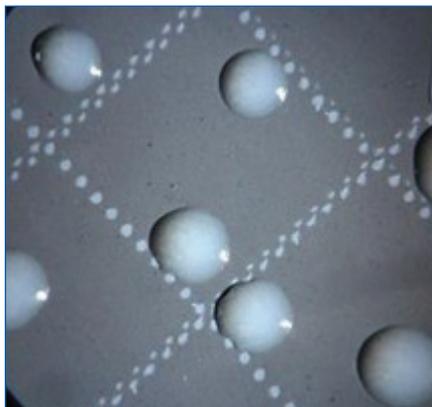
| Membrane Type | Lot | Colony Forming Units <i>Legionella anisa</i> | | | | | |
|----------------|-------|--|---------|---------|---------|---------|--------------------------|
| | | Plate 1 | Plate 2 | Plate 3 | Average | Std Dev | Theoretical Recovery (%) |
| Pall Black PES | Lot 1 | 33 | 40 | 45 | 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 Competitor’s 1 Black NCM membranes, or Competitor’s 2 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 4 days was typical: whitish with a slight ground glass appearance and iridescence (Figure 1). By 7 and 10 days, colonies appeared more beige than white in color and the periphery of the older, larger colonies became slightly transparent (Figure 2). Under UV light, colonies fluoresced blue-white, weakly at 4 days but with greater intensity by 7 days.

Figure 1

Colony morphology on Metrical Black PES membrane

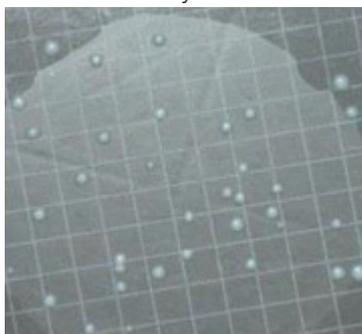


At 4 days, *L. anisa* colonies on the PES membrane filters had typical color and morphology: whitish colonies with slight ground glass appearance and iridescence. Colony growth is not impeded by grid markings

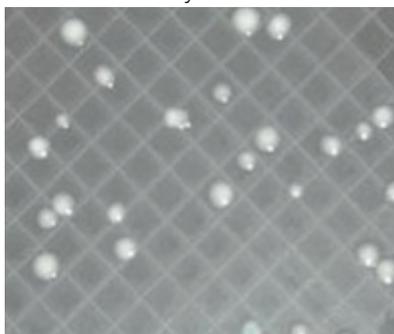
Figure 2

L. anisa colony appearance change from 4 days to 10 days of culture

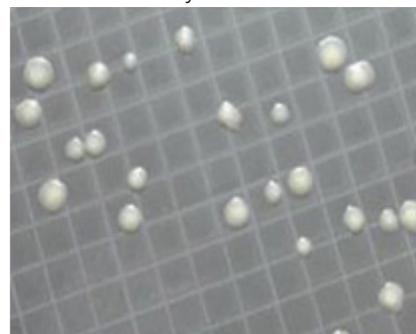
Colonies on Day 4



Colonies on Day 7



Colonies on Day 10



At 4 days, the *L. anisa* colonies on the PES membrane filters were small but visible. Over the 10 days, the colonies grew larger, more beige in color, and their periphery became transparent with less ground glass and iridescence appearance.

Conclusions

In this study performed by a third party laboratory, *L. anisa* was able to grow without apparent inhibition from a pure culture inoculum on all three lots of Pall Metrical black PES membrane filters 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 interlaboratory studies carried out for the validation of the International Standard ISO 11731, "Water quality – Enumeration of *Legionella*." This comparison study confirms the conclusion offered in Annex H of the method that choice of membrane can strongly influence the growth of *L. anisa* and suggests that a polyethersulfone membrane should be considered for recovery of this organism by showing that the use of Pall Metrical Black PES membrane filters for the recovery of *Legionella* in water samples provides more accurate results.



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