PALL Laboratory

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Application Note

Microbiological Evaluation of 0.45 µm Membranes

A Comparative View on Mixed Cellulose Ester (MCE)

Summary

Membrane filtration (MF) technique is the regulatory accepted and preferred method for recovery of microorganisms in products which are filterable. The most accepted filter material for microbiological analysis is mixed cellulose esters (MCE) filter membrane according to the International Pharmacopeias, International Organization for Standardization (ISO) Methods, and the majority of the local standards.

This study looks to quantitatively compare growth observed on Pall Laboratory's GN-6 Metricel® Membrane (MCE) filters with two other MCE membrane filters currently available on the market. Pore size for all the membrane filters were 0.45 µm and had a diameter of 47 mm. All filters were challenged in triplicate using three different microorganisms: *Candida albicans* ATCC # 10231, *Staphylococcus aureus* ATCC # 6538, and *Escherichia coli* ATCC # 8739. These were then plated on solid (agar) media and the results were compared against the control spread plate.

The acceptable range for the calculated percent recovery is 50% - 200% on a membrane filter when compared to a control spread plate and a pattern between the three membranes tested is clearly shown. All membrane filters performed very similarly and showed no significant difference in recovery hence, there is minimal difference between the three MCE membrane filters.

Materials and Methods

The pore size and membrane material selection used in this study were chosen based on accepted membrane filter applications as outlined by international standards.

The membrane filters used were tested aseptically inside of an ISO Class 5 workstation. Each filter was inoculated with 1-100 colony forming units (CFU) of each of the challenge microorganisms and then plated onto media optimum for each microorganism's growth. After inoculation, the plates containing the filters were incubated at the optimum growth temperature for each of the challenge microorganisms and observed each day after incubation for growth.

Each filter cup was rinsed with 50 mL of 0.9% sterile saline, inoculated with one of the challenge in triplicate and followed by another rinse of 50 mL of 0.9% sterile saline. After filtering, each membrane was transferred aseptically to the media plate appropriate for each organism. All bacterial positive controls, negative controls, and samples plates were incubated for no more than 3 days with their respective temperatures (Table 1).

Table 1

Filter Media used for Organisms

Organism	Media Plate Used	Temperature of Incubation
Escherichia coli ATCC # 8739	TSA	32.5 ± 2.5 ºC
Staphylococcus aureus ATCC # 6538	TSA	32.5 ± 2.5 ºC
Candida albicans ATCC # 10231	SDA	22.5 ± 2.5 ºC

Results

Three different microorganisms were tested to compare growth quantitatively in triplicates using the membrane filtration technique. Once the colonies were counted and recorded, the percent recovery of the organisms were calculated based on the positive control spread plate.

The results seen here are intended to give a broad overview of how Pall Laboratory's MCE membrane performs against two other competitor membranes. Based on these results, we can see that all membranes demonstrated acceptable recovery for all membranes tested.

The following tables and graphs show the average plate count with 0.45 µm pore size of all the membranes tested with the three microorganisms chosen.

Table 2

Spread Plate Evaluation

Organism	Plate Counts (CFU)		Average Plate Count (CFU)
Escherichia coli ATCC # 8739	65	38	52
Staphylococcus aureus ATCC # 6538	49	65	57
Candida albicans ATCC # 10231	63	57	60

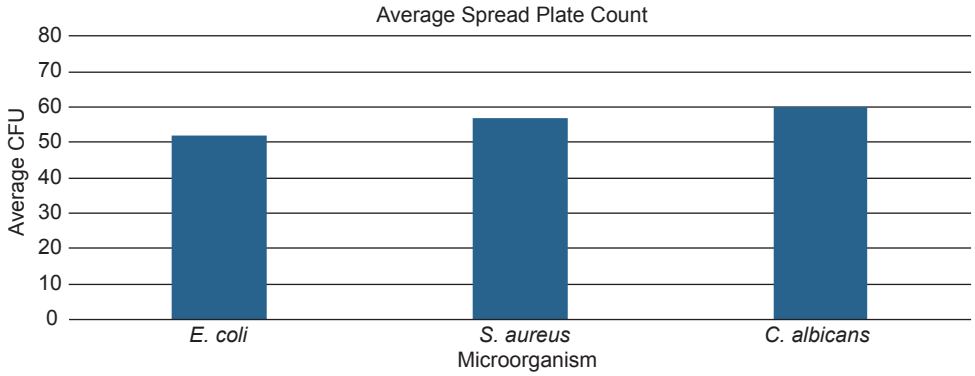


Table 3

Challenge Organism: E. coli

Filter Type	Plate Counts (CFU)			Average Plate Count (CFU)	Percent Recovery
Pall	45	52	48	48	92%
Competitor 1	51	38	50	46	88%
Competitor 2	45	39	49	44	85%

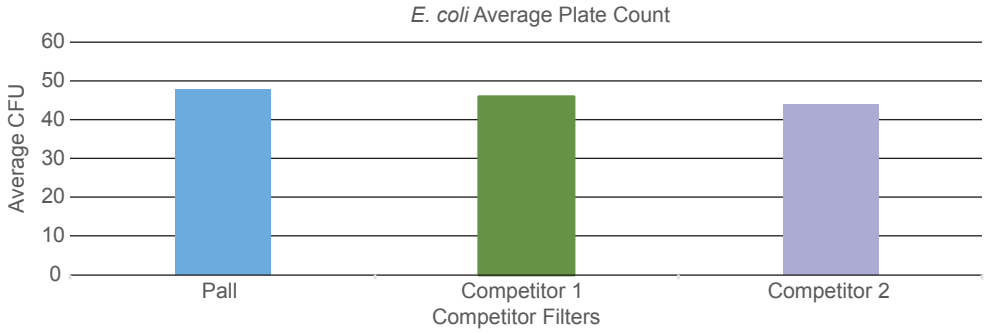
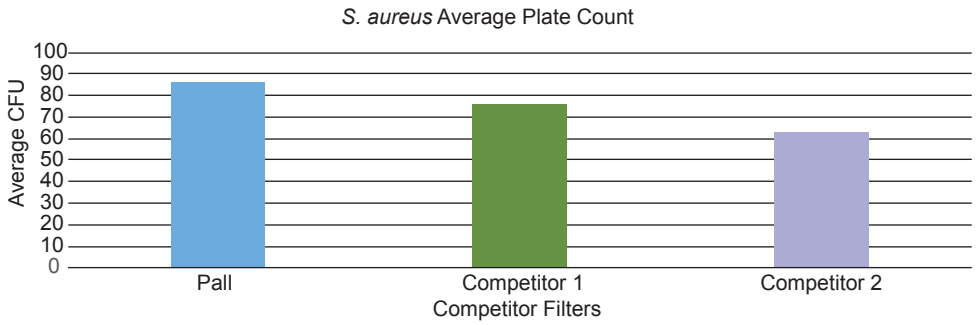
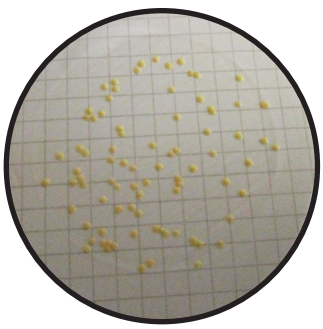


Table 4

Challenge Organism: S. aureus

Filter Type	Plate Counts (CFU)			Average Plate Count (CFU)	Percent Recovery
Pall	74	88	96	86	151%
Competitor 1	79	73	75	76	133%
Competitor 2	64	64	60	63	111%



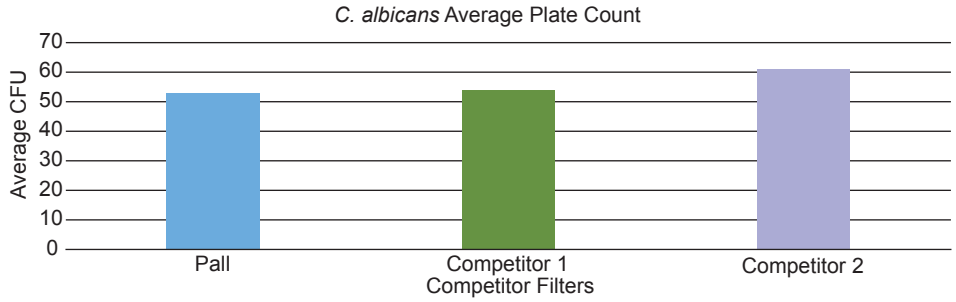


Membrane Filtration Growth:
Pall *S.aureus*

Table 5

Challenge Organism: C. albicans

Filter Type	Plate Counts (CFU)			Average Plate Count (CFU)	Percent Recovery
Pall	41	67	52	53	88%
Competitor 1	55	51	55	54	90%
Competitor 2	63	57	63	61	102%



Conclusion

As membrane filtration is the method of choice for all microbiology testing, it is imperative that a study is done to demonstrate recovery versus a control plate. By testing in triplicate and comparing the average result to the control plate, a clear pattern of similarity is seen where all three membranes performed within the acceptance criteria.

Because all the membranes performed statistically similar, the verification process from one membrane to another membrane may not be as time consuming and difficult as initially thought. It is recommended to consult with your own internal Regulatory Affairs and Quality Assurance groups before making any changes to your test methods.

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