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Note

Sterilization performance comparison between an autosampler-ready microporous filter vial and a syringe-based filter

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ABSTRACT

Herein we report that the Thomson Standard and eXtreme/FV® filter vials (0.2 µm polyvinylidene difluoride filter membrane) are as effective as gold standard microporous membrane-based syringe filters at removing bacteria, such as *Klebsiella pneumonia,* from media samples produced in the microbiological laboratory.

As automated sampling technologies (e.g., autosampler mediated sampling during liquid chromatography (LC)-fluorescence, UV-VIS, or tandem mass spectrometry measurements) increase in prevalence in the microbiology research laboratory, so too will the demand for high-throughput sample-processing technologies that are adaptable to the rigors of microbiological workflows (Jannetto and Fitzgerald, 2016; Huang et al., 2014). Prior to analysis, bacteria-containing samples should be filter sterilized to reduce pathogenicity and enhance method robustness (i.e., analytical column longevity) (Stoll, 2017). One area where handling, time, and cost-saving improvements may yield immediate research dividends is in the post-processing of media sample fractions that have been inoculated with pathogenic organisms, such as *Klebsiella pneumoniae* (*K. pneumoniae*) or *Staphylococcus aureus*. Currently, the state-of-the-art in microporous filter-based sterilization methods use either a syringe-based format for small sample sets, or a 96-well based format for larger sample sets (Bobbitt and Betts, 1992). Either format may require several or all of the following procedural steps: i) bacteria-containing media is aspirated into a syringe, or a pipette tip for 96-well applications; ii) for syringe filter applications, a sterile filter is installed on the barrel of the syringe, and for 96-well applications, the sample is transferred into an individual well in a 96-well plate; iii) media is filtered via positive mechanical pressure that is applied to the plunger of the syringe, or by compressed nitrogen gas directed to the 96-well plate via a manifold; iv) filter sterilized media samples are collected in sterile tubes or plates; v) Internal Standard and enzyme quenching solvents are added to individual samples; and, vi) samples are transferred into autosampler injection vials.

Within the last 5 years, a series of commercially available, autosampler vial-based microporous filtration devices have been introduced that have the potential to reduce steps ii-vi above into a single processing step (See Fig. 1). The device is comprised of a small polypropylene shell where the bacteria-containing media sample, internal standard, and any additional enzyme-quenching/bactericidal solvent system is added. Then, a plunger device that contains an integrated microporous filter membrane is installed and depressed. The bacteria-free media filtrate accumulates in the void of the plunger device and the filter-sterilized media sample is available for injection by an autosampler – non-viable bacteria remain sandwiched in the interstitial space between the shell and the plunger. A short video illustrating the use of the autosampler-ready microporous filtration device has been recorded, and accompanies the electronic version of this manuscript. Click on the image below to access the video. Herein, we present side-by-side comparisons of the effectiveness of the filter sterilization capabilities of the Standard (single layer) and eXtreme/FV® (multi-layer) autosampler vial-based filtration devices against a gold-standard syringe-based microporous filter device. The test organism used for this performance assessment was a wild-type *K. pneumoniae* (KP-1427) bacterial strain.

The KP 1427 WT strain of *K. pneumoniae* was from ATCC (Manassas, VA). Olympus syringe filters (13 mm, 0.22 µm polyethersulfone (PES))

Abbreviations: LC, liquid chromatography; UV–Vis, ultraviolet and visible spectrophotometry; *K. pneumoniae*, *Klebsiella pneumoniae*; PES, polyethersulfone; PVDF, polyvinylidene difluoride; PBS, phosphate buffered saline; CFU, colony forming units; OD, optical density

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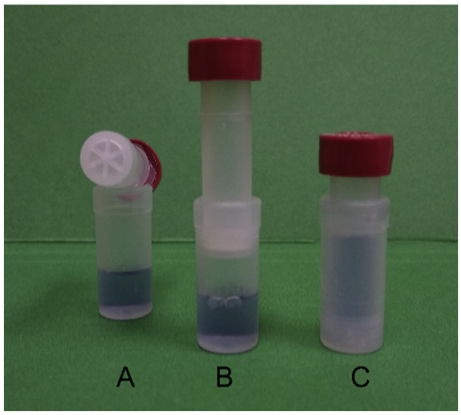

Fig. 1. This figure illustrates the two components comprised in an autosampler vial-based microporous filtration device. Panel A shows the plunger portion of the device (the filter can be observed inside of the pie-shaped lattice) lying across the top of the shell portion of the device that contains a 400 µL volume of MacConkey Broth – the integrated filter is visible between the pie-shaped lattice. Panel B shows the device assembly prior to filter sterilization of the MacConkey broth. Panel C shows the device assembly after the MacConkey Broth sample has been filter sterilized - the sample is ready for LC analysis.

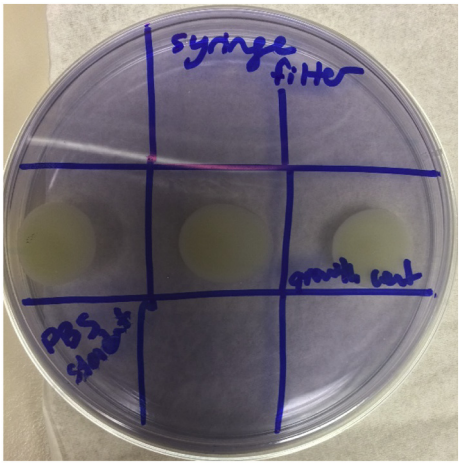

Fig. 2. The photograph of the first MacConkey Agar plate contains the following sample sets with three replicates for each type: Top row: Olympus (0.22 µm PES) filter-sterilized samples; Middle Row: non-filtered positive bacterial growth control samples; and, Bottom Row: bacteria-free negative growth control collected from the Thomson Standard filter vial.

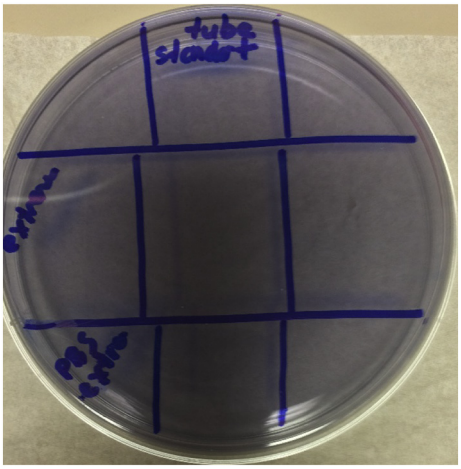

Fig. 3. The photograph of the second MacConkey Agar plate contains the following sample sets with three replicates for each type: Top row: Thomson Standard filter vial-sterilized samples; Middle Row: Thomson eXtreme/FV® filter vial-sterilized samples; and, Bottom Row: bacteria-free negative growth control collected from the Thomson eXtreme/FV® filter vial.

were from Genesee Scientific (San Diego, CA). Standard and eXtreme/FV® autosampler filter vials (0.2 µm polyvinylidene difluoride (PVDF)) were from Thomson Instrument Company (Oceanside, CA). MacConkey broth, agar, and sterile phosphate buffered saline (PBS) were from VWR (Radnor, PA).

A petri dish containing MacConkey Agar (MacConkey Agar plate) was inoculated with *K. pneumoniae* bacteria using a four-quadrant method, and incubated aerobically overnight at 37 °C. Bacterial growth and purity was assessed prior to experiment initiation. A bacterial suspension was prepared in sterile PBS at a bacterial cell density of 6.6×10^8 CFU/mL (OD of 0.483 at 600 nm).

Four individual bacteria samples were prepared by diluting 100 µL of the bacterial suspension in 500 µL of sterile PBS, and each sample was gently vortex-mixed for 10 s. The individual samples were processed as follows: i) one sample was left unfiltered to act as a positive growth control; ii) a 200 µL volume of a sample was filter sterilized using a sterile Olympus 0.22 µm PES syringe filter; iii) a 250 µL volume of a sample was filter sterilized using a non-sterile Thomson Standard 0.2 µm PVDF filter vial; and, iv) a 250 µL volume of a sample was filter sterilized using a non-sterile Thomson eXtreme/FV® 0.2 µm PVDF filter vial. Negative growth control samples were prepared in order to assess the presence of bacterial contaminates on the surfaces and filter membranes of the Standard and eXtreme/FV® filter vials because a sterilized option does not yet exist for these devices. The exterior surface area of two separate MacConkey Agar plates was sub-divided into three rows and three columns – the rows and columns each corresponded to individual sample types, and technical replicates, respectively. After processing, three 10 µL sample volumes from each processed condition were blotted onto pre-designated zones of the MacConkey Agar plates, and each was incubated aerobically overnight at 37 °C.

After 18 h of incubation, the two MacConkey Agar plates were removed from the incubator and visually assessed for the presence of bacterial colony growth. As expected, there were large mucoid colonies of *K. pneumonia* present for the three replicates of the non-filtered positive growth control samples (Fig. 2 – middle row). No bacterial growth was evident for the three replicates of the syringe filter sterilized bacterial samples (Fig. 2 – top row), or for the three replicates of each for the Standard (Fig. 3 – Top row) and eXtreme/FV® (Fig. 3 – middle row) filter sterilized bacterial samples. Additionally, there was no evidence of bacterial cross-contamination for the two filter vial sterilized bacterial samples, or from each of the negative growth controls (Standard, Fig. 2 – bottom row; eXtreme/FV®, Fig. 3, bottom row).

Herein, experimental data have been provided that shows that the Thomson Standard and eXtreme/FV® filter vials were as effective at removing *K. pneumonia* bacteria from bacteria-containing PBS samples as a gold-standard syringe filter-based sterilization system, such as the Olympus 0.22-µm PES filter membrane. Additionally, the absence of colonies present in the negative growth control samples for both filter vials tested suggests that there was no bacterial contamination on the devices – however, only sterile devices and solutions are typically used in the microbiology lab setting in order to prevent bacterial cross contamination.

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References

Bobbitt, J.A., Betts, R.P., 1992. The removal of bacteria from solutions by membrane filtration. J. Microbiol. Methods 16 (3), 215–220. https://doi.org/10.1016/0167-7012(92)90006-P.

Huang, L., Haagensen, J., Verotta, D., Lizak, P., Aweeka, F., Yang, K., 2014. Determination of meropenem in bacterial media by LC-MS/MS. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 981, 71–76. https://doi.org/10.1016/j.jchromb.2014.05.002.

Jannetto, P.J., Fitzgerald, R.L., 2016. Effective use of mass spectrometry in the clinical laboratory. Clin. Chem. 62 (1), 92–98. https://doi.org/10.1373/clinchem.2015.248146.

Stoll, D.R., 2017. Filters and filtration in liquid chromatography-what to do. LCGC N. Am. 35 (2), 98–103.