Technical Brief

Top Loading Liquid Media to the 55-Plus™ Monitor Increases Efficiency and Saves Time

The 55-Plus Monitor is a fast and high throughput filtration device for detection and enumeration of microorganisms in beverages.

The 55-Plus Monitor is a disposable filtration unit designed for the detection and enumeration of microorganisms in beverages. This easy-to-use product allows beverage manufacturers to detect microbial contamination in sample liquids such as final product or during in-process testing. The 55-Plus Monitor was originally designed for the operator to add the liquid media through the bottom port in the base. In order to do this, the operator inverts the monitor and expels the liquid media from an ampoule into the base from the bottom port. This operation is frequently awkward and messy. In this technical brief, we demonstrate that it is possible for the operator to use an alternate method, (top loading), to introduce the liquid media, achieving results that are equivalent to the standard (bottom-loading) method. Top loading allows the operator to add the liquid media directly into the top of the filtration funnel and then apply vacuum to absorb the media into the pad. This method removes the need to invert the monitor and wait for the media to reach the pad.

TOP-LOADING STUDY OBJECTIVES

This technical brief highlights the study performed at Millipore to determine if top loading liquid media to the absorbent pad increased efficiency and provided results that were at least equivalent to the bottom-loading method. The results of this study indicate that top loading is a valid, acceptable procedure for adding liquid media to the 55-Plus Monitor.

MATERIALS AND METHODS

Monitors

One lot of 55-Plus Monitors (catalogue number JBRMHWG05505) was used on a Millipore six-tulip filtration manifold (catalogue number XX25 047 00) that was connected to a Millipore vacuum pump with a 1 liter trap and a Millipore Millex® FG filter to prevent contamination of the pump with liquid.

Culture Media

The study used the following media:

- m-HPC broth (Millipore catalogue number MHA000P2S)
- m-Endo broth (Millipore catalogue number MHA000P2E)
- m-Green Yeast and Mold broth (Millipore catalogue number MHA000P2M)

Microorganisms

The targeted colony count was 10 to 100 CFUs (colony forming units) for the monitors using the top-loading method as well as for the control test monitors using the bottom-loading method. The study used the following microorganisms:

- *Escherichia coli* (ATCC 25922)
- *Pseudomonas aeruginosa* (ATCC 27853)
- *Staphylococcus aureus* (ATCC 6538)
- *Aspergillus niger* (ATCC 16404)
- *Saccharomyces cerevisiae* (ATCC 7754)
- *Citrobacter freundii* (ATCC 8090)
- Orange juice concentrate (grocery store brand), reconstituted and incubated for 24 hours at 30 °C, containing natural beverage microbial contaminants (yeast, mold, bacteria).
- Primary effluent from a wastewater treatment plant in Concord, MA.

SAMPLE PREPARATION, FILTRATION, AND INCUBATION

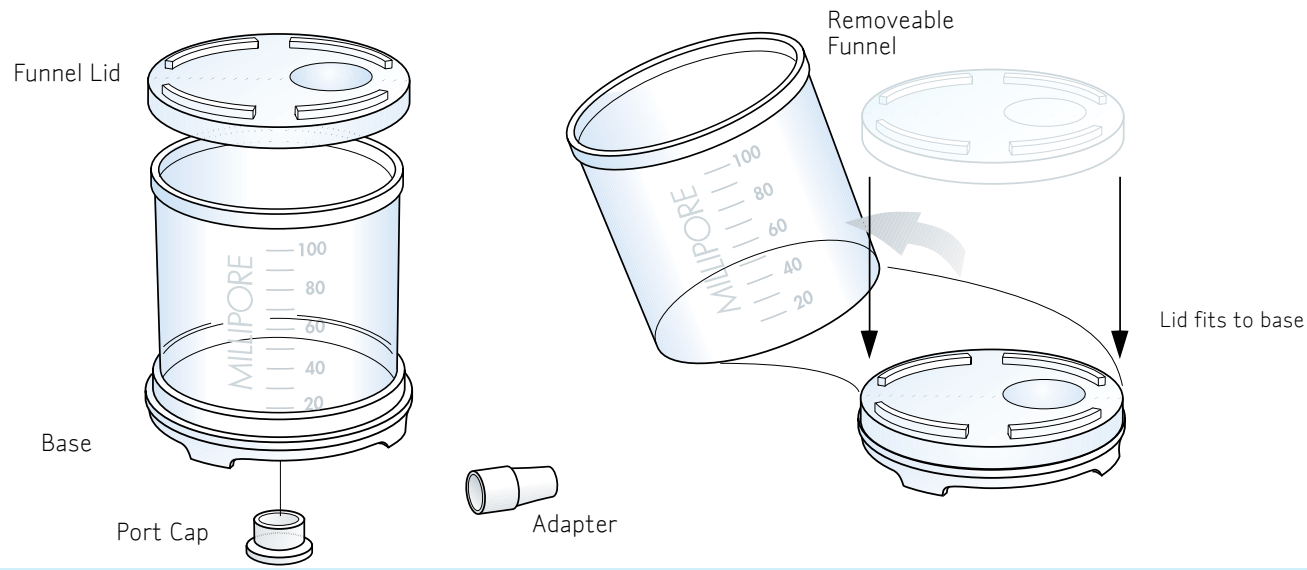
To prepare the sample, *E. coli, P. aeruginosa, S. aureus, and C. freundii* were each inoculated into Soybean-Casein Digest broth for 24 hours at 35 ±2 °C ; *S. cerevisiae* and *A. niger* were grown for 72 hours at 25 ±2 °C. Each microorganism and natural sample was diluted to yield 10 to 100 CFUs per monitor and each sample was filtered using 0.1% peptone water as instructed by the manufacturer.

The study tested each sample in replicates of four monitors per medium. In the control set, one medium was added to the bottom of the membrane through the port under the pad; in the test set, one medium was added to the top of the membrane and the vacuum applied for 1–2 seconds to force the medium into the absorbent pad. Samples tested with m-Endo and m-HPC broth were incubated at 35 ±0.2 °C for 24 hours; samples tested with m-Green Yeast and Mold broth were incubated at 25 ±2 °C for 72 hours. Colonies were counted on both the test and the control monitor membranes with an average for each set calculated. Percent recovery was calculated by:

$$\% \text{ recovery} = \left[\frac{\text{average \# cfu (test set)}}{\text{average \# cfu (control set)}} \right] \times 100$$

The acceptance criteria for the test (top-loading) method was ≥85% recovery when compared to the results of the control (bottom-loading) method.

The 55-Plus Monitor – Easy-to-use, Sterilized Disposable Filtration Device



The monitor is composed of three pieces. There is a base with a mixed cellulose ester membrane bonded to an inert cellulose pad (to hold the liquid culture medium in contact with the membrane filter). On the underside of the base is a port to connect the monitor to a vacuum adapter – in a rubber stopper on a vacuum flask or vacuum manifold. Above the base is a removable graduated funnel to measure and hold the sample. The monitor has a lid that helps minimize contamination during filtration and covers the filter during incubation.

DATA INTERPRETATION

The study demonstrated that adding liquid media through the top of the 55-Plus Monitor is as effective as adding the media through the bottom port. In the m-HPC set, an average of 104% recovery was calculated (figure 1). With m-Endo, the average recovery was 113% (figure 2), while with m-Green Yeast and Mold broth, recovery was 95% (figure 3).

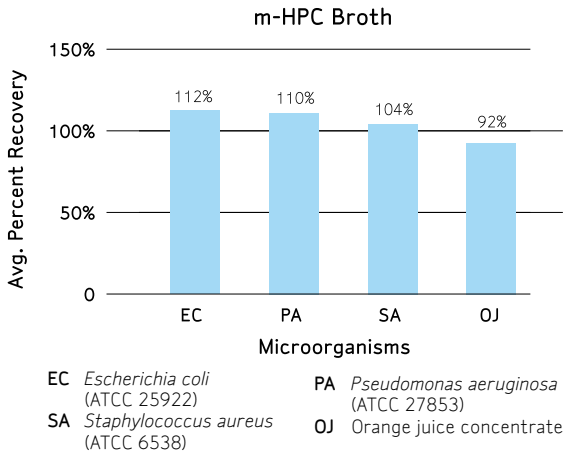
The average colony counts were analyzed for each microorganism on each medium for the top-loaded (test) monitors and the bottom-loaded (control) monitors. The percent recovery (average test counts or average control counts) for each type of medium appears in the figures. The figures demonstrate the average percent recovery of top loaded (test group) vs. bottom loaded (control).

The average recovery for all three media with the test top-loaded monitors was 104% when compared to the control bottom-loaded monitors. After reviewing the data, the study determined (through a Student T Test) that there was no significant statistical difference between the results of the test and control monitors.

CONCLUSION

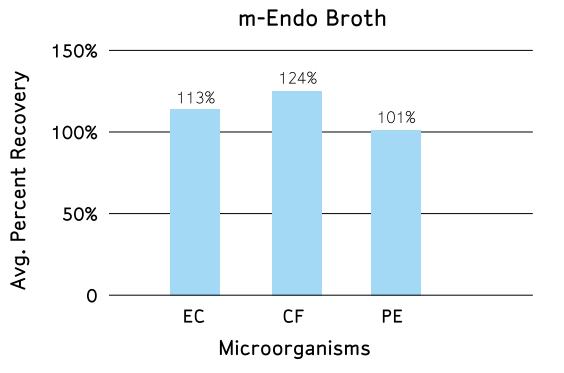
The study demonstrated there was no statistically significant difference in recovery between the top-loading and bottom-loading methods. In the past, Millipore recommended bottom loading the liquid media to increase the microorganism recovery. The results of this study show that there is no advantage to bottom loading versus top loading. The study also found that operators load the media faster and more efficiently using the top-loading method. Therefore, based on this study, accurate results can be achieved using either the top or bottom loading method, and top-loading can improve efficiency by decreasing time spent adding media.

Results of Top Loading Media Compared to Bottom Loading Media (Control)



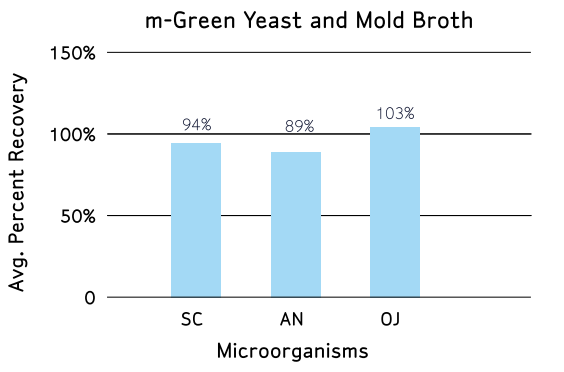
EC *Escherichia coli* (ATCC 25922)
SA *Staphylococcus aureus* (ATCC 6538)
PA *Pseudomonas aeruginosa* (ATCC 27853)
OJ Orange juice concentrate

Figure 1. Average percent recovery for m-HPC Broth



EC *Escherichia coli* (ATCC 25922)
CF *Citrobacter freundii* (ATCC 8090)
PE Primary Effluent

Figure 2. Average percent recovery for m-Endo Broth



SC *Saccharomyces cerevisiae* (ATCC 7754)
AN *Aspergillus niger* (ATCC 16404)
OJ Orange juice concentrate

Figure 3. Average percent recovery for m-Green Yeast & Mold Broth

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