cytiva

Membrane filtration for water quality testing

cytiva.com

The membrane filtration (MF) technique in microbiology

In the early 1950's, membrane filters gained a large audience in bacteriology for their ability to concentrate particulate and cells on the surface. The bacteria captured on the surface of the filter could be grown into individual colonies when the membrane was placed face-up on a suitable growth medium (Fig 1). As nutrient media were further developed and specialized, selective growth media became available and the technique grew in importance in the field of sanitary bacteriology — water and wastewater quality monitoring. By the middle of the decade, many researchers were publishing data on coliform recovery from water using membrane filters, and the technique became accepted as a valuable standard method for water quality testing. However, there were also numerous publications focused on aspects of the membrane filters that could distinguish performance, as well as performance limitations with certain organisms. Those challenges still exist today as membrane manufacturers strive to optimize and balance membrane performance across a broad range of organisms. It is also what drives method development groups such as the International Organization for Standardization (ISO) to scrutinize membrane performance for particular waterborne or water quality indicator organisms with and/or without specialized growth media.

(A) (B) (C)

Fig 1. Membrane filtration workflow. (A) Secure membrane and funnel; (B) Filter and rinse sample; (C) Retrieve and plate membrane. The MF technique provides a simple method to concentrate and culture bacteria from water samples for water quality monitoring.

Benefits of MF technique

MF technique was recognized early on as an ideal way to process water quality samples for analysis of microbial content for the following reasons:

- **Improved sensitivity**: allows for concentration of the content of larger sample volumes onto the surface of a membrane filter.
- **Separation from inhibitory substances:** organisms are captured on the membrane and any substances dissolved in the water, such as chlorine compounds or heavy metals, will flow through the membrane.
- **Neutralization of inhibitory substances:** the use of rinse buffers also acts to rinse away and neutralize substances that could impede or inhibit organism growth.
- **Isolated colonies:** the resulting growth on the membrane surface develops into discrete colonies that can be easily counted and selected for further characterization or identification, if necessary.

Membrane filter selection

Membrane filters are offered in a range of pore sizes, colors and packaging varieties (Fig 2). Selection of pore size may be called out specifically in some standard methods while others suggest a suitable pore size range. In the instance where the technician is offered a range, pore size is selected to provide the optimal recovery of the target organism(s) balanced by the desired flow rate to filter the sample efficiently. Various membrane filter color options are also available. The colors provide contrast of the recovered colonies against the membrane surface, allowing accurate identification and quantification. The selection of a white, black or green membrane would be based on the target organism, the type of specialized selective growth media used, and whether that media induces a colony color to develop that would stand out better against a white or colored background.

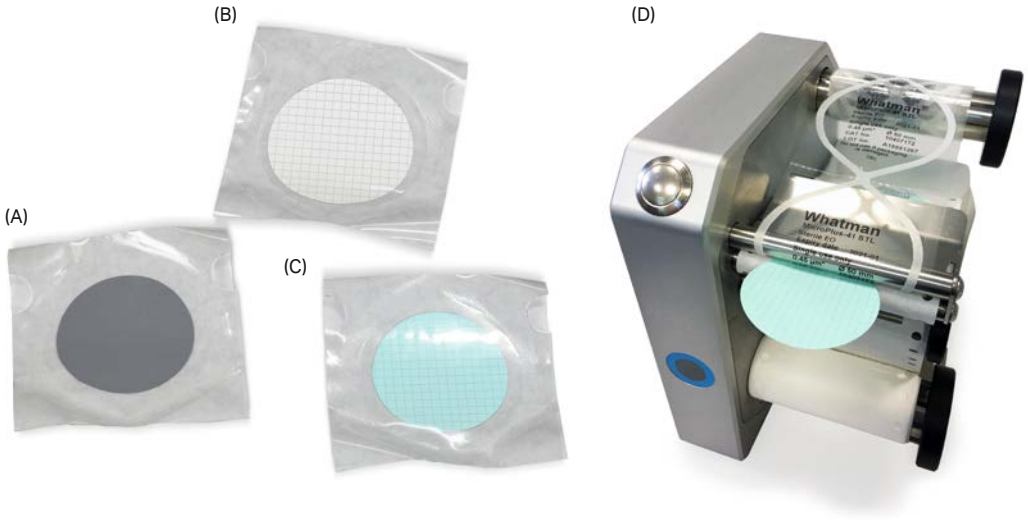


Fig 2. Whatman membrane filters are available in a (A) black, (B) white and (C) green, in a range of pore sizes from 0.2 to 3.0 µm. Shown here is individual sterile packs, they are also available in an (D) eButler dispenser pack for automated dispensing.

Membrane properties make a difference

Within many laboratory techniques there are various operating and material parameters that affect the quality of results. The most important influence on the accuracy of results obtained with the MF technique is, as expected, the quality of the membrane used.

It is important to consider many membrane properties when selecting the best and most suitable membrane to use in your microbiological analysis.

1. Colony recoveries

The most important aspect of membrane performance is the ability to recover a high percentage of colonies from the test inoculum. High recovery performance of the test culture should also reflect high recovery performance of an unknown quantity of test organisms in a sample. Colony recoveries are tested by inoculating a known range of organisms to a series of membrane samples and comparing colony counts to a culture density check applied directly to the growth media. Due to early adoption of the MF technique into water quality methods which already used coliforms and *E. coli* as targets, those indicator organisms were also adopted by membrane manufacturers for quality control testing of in-process and finished product membrane filters for microbiological monitoring. Over the years, methods have been adapted for using the MF technique for other indicator and/or pathogenic organisms, with increased guidance for qualifying membranes for a broader range of organisms.

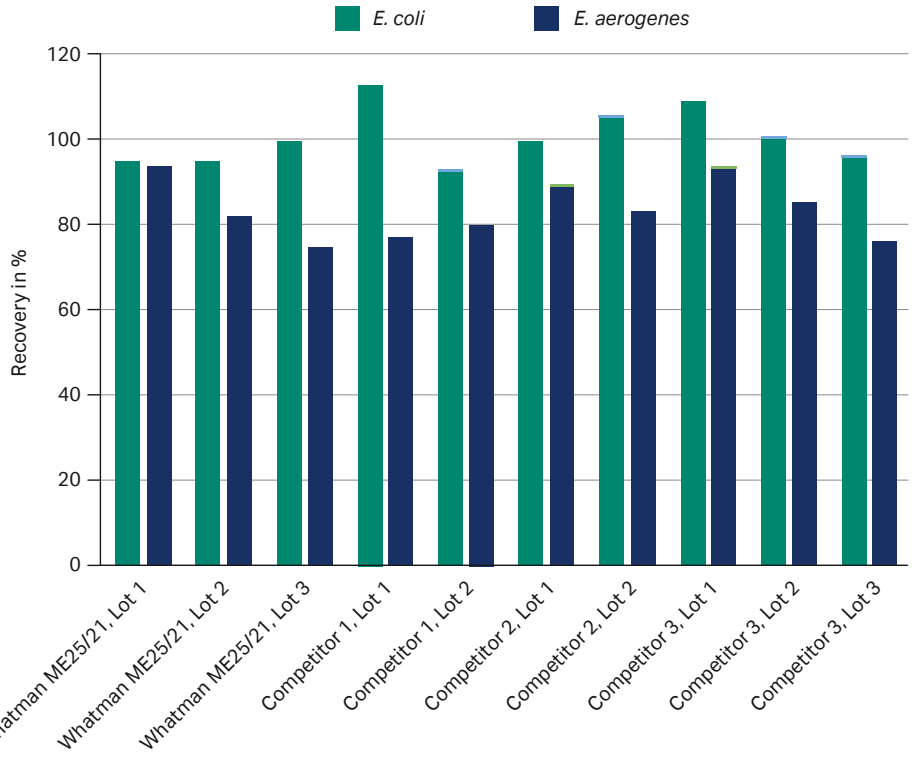


Fig 3. Recovery performance expressed as percent of colonies that grew on the membrane as compared to direct inoculation of an agar plate. Test method designed in accordance with ISO 7704, ISO 8199, ISO 11133 guidance. Test organisms *Escherichia coli* WDCM00179, Enterobacter aerogenes WDCM00175; test media: chromogenic coliform agar (CCA); reference media: TSA Agar; incubation time: 21–24 h at 36 ± 2 ºC.

2. Colony morphology

Colony morphology, the appearance of the colonies as they grow on the filter media, is closely tied to membrane recoveries and performance. Each method specifies incubation conditions —time, temperature, media—and has expectations of the typical colony characteristics the target organism will exhibit. These characteristics include color, size, shape and edge type (Fig 4). Many of the methods call out selective media that are formulated to promote the growth of the target organism while suppressing the growth of non-target organisms. The medium may also be selective in that the ingredients may cause the target organism to grow with a characteristic pigmentation, take up a dye, or cause an enzymatic reaction specific to that group of organisms. Some methods are particular about stating a time and temperature range which may also influence proper development of colony morphology, so the target organisms can be accurately counted. Colony size may come into consideration if the samples have been properly incubated within the prescribed time but continue to be particularly small, which may indicate inhibition of growth, or grow particularly large, which could indicate enhancement of growth. Enhancement of growth becomes a problem if there is a possibility of non-target organisms in a real test sample competing for nutrients and causing suppression or masking of the target organism growth, leading to inaccurate results.

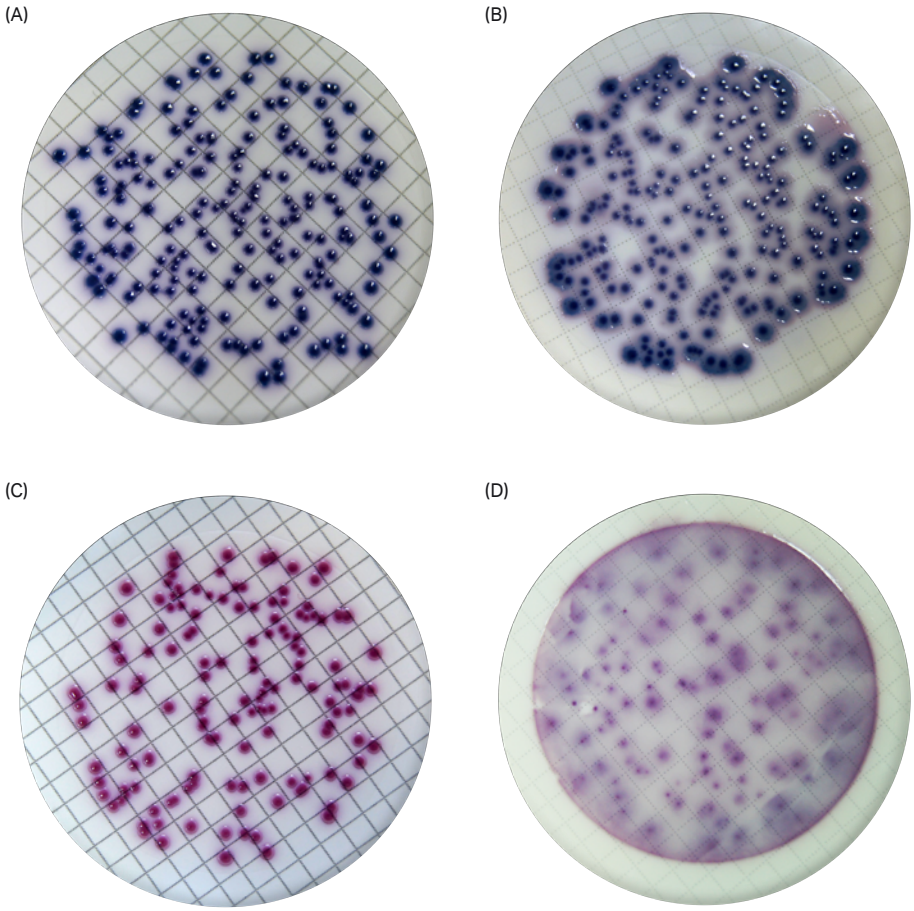


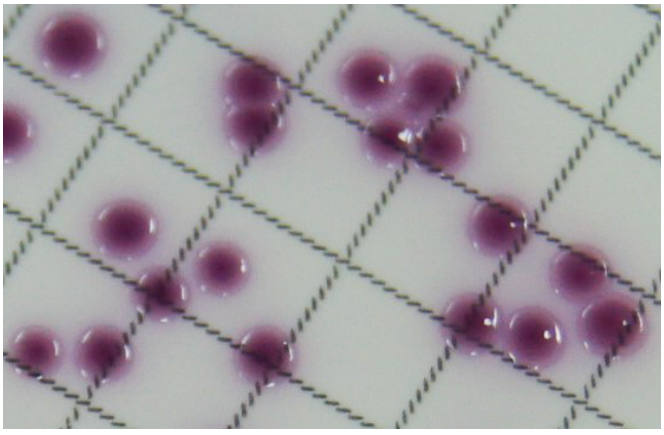
Fig 4. Comparison of acceptable v. unacceptable colony morphology of (A and B) *Escherichia coli* and *(C and D) Enterobacter aerogenes* cultured on CCA medium. (A) and (C): Clear edges and consistent shape and size indicate good morphology, enabling accurate colony count. (B) and (D): Poor morphology, indicated by irregular size, spreading edges and coalescing colonies.

3. Grid lines

Grid lines on microbiology membranes facilitate the counting of colonies growing on the surface of the membrane. The grid lines should not inhibit, enhance, or disrupt the normal morphology of the target organisms. Inhibition may be recognized as colonies exhibiting a narrow, flattened, or squared-off appearance as it grows against or across the grid line. Other undesirable growth characteristics may include trailing growth along the grid line with no discernible colony distinction (Fig 5).

Using grid lines as a counting guide: If colony density is high, grid lines allow a way to estimate colony growth by counting a prescribed number of squares and multiplying by the total squares in the effective filtration area (EFA). The design and pattern of the grid line is an important consideration. It is generally accepted that a broken line pattern allows colonies to grow uninhibited against and across the grid line, while solid lines may have a higher likelihood of creating problems. In addition, the heavier application of ink to form a solid pattern line creates more of a contrast and potential eye-strain, especially when examining plates under 10x magnification.

(A)



(B)

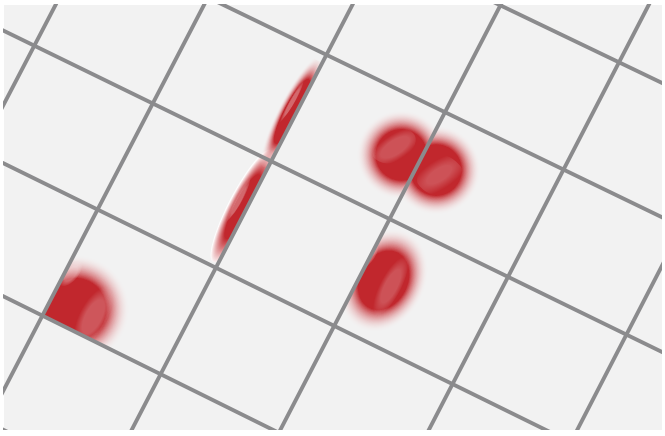


Fig 5. Grid line influence on colony growth. (A) Normal colony growth across grid lines. (B) Representation of distorted colony growth where colonies meet grid lines.

4. Wettability

Membrane wettability is the ability of a liquid to wet the membrane structure. The wettability of a membrane is based on the chemical properties of the membrane surface. Typically, the polymers used to produce microporous membranes are hydrophobic in nature, meaning they will not wet out with water. There are, however, some naturally hydrophilic materials that may be used in membrane production, for example nylon and cellulose.

Wettability is of concern for the microbiological quality control of membranes for two main reasons. First, the instant and thorough wetting of the membranes will prevent uneven distribution of the colonies during the filtration. Complete wetting of the membrane should provide an even distribution of organisms across the effective filtration area. Second, a thoroughly wetted membrane with no hydrophobic spots will provide for better uptake and transmission of nutrients to the organisms on the surface of the membrane, allowing for normal growth and morphology.

Wettability may be assessed by floating a membrane disc on the surface of water and observing the membrane darken evenly as it wets. Persistent white spots, speckles, diffuse areas or persistent whiteness along gridlines indicate areas that are not wetting properly and may remain hydrophobic through filtration and incubation. Filtering a light slurry of fine carbon particles can help visualize the distribution across the effective filtration area, as shown in Figure 6. This method can show patterns of particle distribution caused by membrane irregularities such as hydrophobic spots or influences of the funnel support screen or grid line.

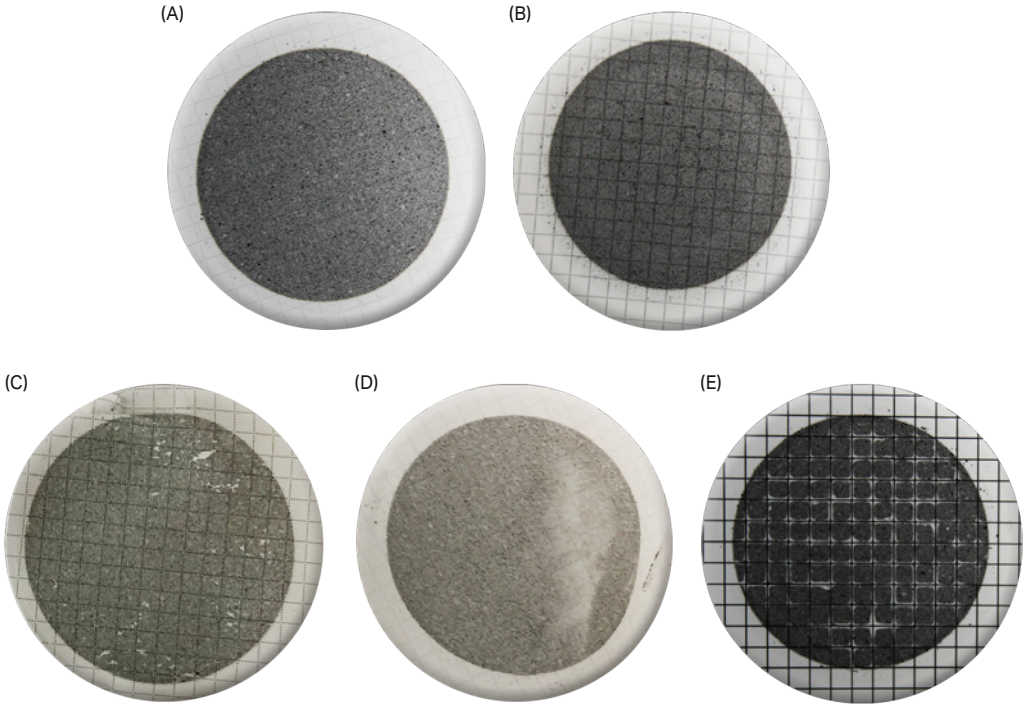


Fig 6. Charcoal deposition. (A) and (B) show even deposition of charcoal, indicating consistent hydrophilic properties across the membrane. (C) Random hydrophobic spots and (D) diffuse area of hydrophobicity (D) indicate inconsistent wetting. (E) shows grid line influence on wettability.

5. Retention

Membrane retention is the ability of the filter to prevent the passage of organisms through the membrane. A heavy suspension of test organism is filtered through the membrane filter until the membrane has experienced a concentration of 10^7 organisms/cm². The filter is expected to retain all of the challenge organisms. In water quality testing, retention performance is characterized for 0.45 µm filters using a challenge of *Serratia marcescens*. Below is a table of the generally accepted test organism by pore size.

Table 1. Specifications for membrane retention testing

Pore size	Test organism	Concentration	Basis norm
0.15	*Burkholderia cepacia* (ATCC 17770, DSM 50180)	10^7/cm²	DIN 58355-3:2005; ASTM D3862-13
0.2	*Brevundimonas diminuta* (ATCC 19146, DSM 1635)	10^7/cm²	DIN 58355-3:2005; ASTM D3862-13
0.45	*Serratia marcescens* (ATCC 14756, DSM 1636)	10^7/cm²	DIN 58355-3:2005; ASTM D3862-13

6. Flow rate

While flow rate used to be a described membrane characteristic, the current standard methodologies opt for more general characterization, for example, Standard Methods, 23rd edition, 9222 g. "a satisfactory filtration speed (within 5 min)." Most manufacturers have developed their microbiological membranes around a typical range. The importance to the analyst is what that means for their sample filtration time and whether that affects their workflow as they process their samples. The mean flow rates for commonly available membrane filters are shown in Figure 7.

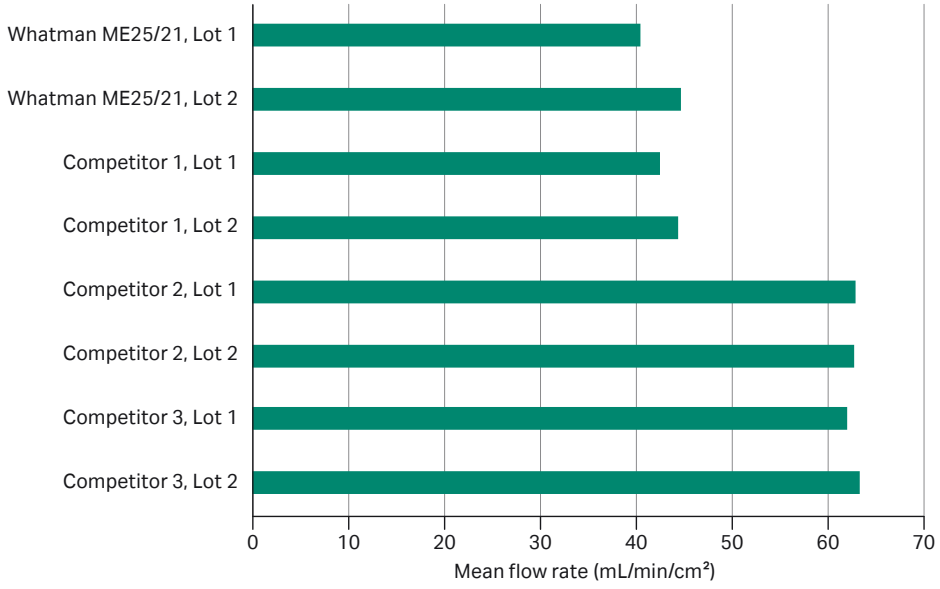


Fig 7. Typical flow rates of competitive grades of white, 0.45 µm membrane filters measured in milliliters per minute per centimeter squared filtration media.

7. Thickness, pliability, brittleness

While thickness can easily be measured and compared between manufacturers, as shown in Figure 8, it cannot be used in isolation as a predictor of membrane handling characteristics. Thickness, pliability and brittleness all act together to create handling characteristics. Membrane ease of use and good handling performance are subjective from analyst to analyst. Often, slight modifications to the technique with which the analyst handles the membrane can overcome issues. For example, using round-tip, blunt forceps to handle the membrane rather than pointy or serrated-tip forceps can prevent tearing of the filter. Using a rolling motion to remove the filter up from the test stand rather than pulling or dragging the filter off the surface in a horizontal motion can provide easier removal with less risk of tearing.

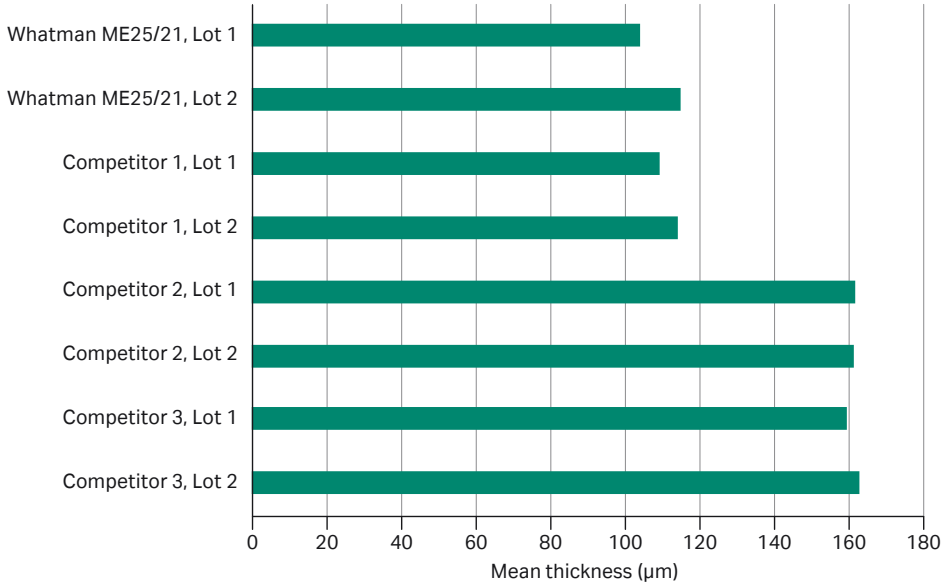


Fig 8. Typical thickness of competitive grades of white, 0.45 µm membrane filters measured in µm.

Choosing the right membrane filter

The MF technique is an effective and accepted technique for testing fluid samples for microbiological contamination. It involves less preparation and culture media than many traditional methods, and is one of a few methods that will allow the isolation and enumeration of microorganisms.

Membrane filters are available in a range of pore sizes and colors. Physical properties of membranes can vary from supplier to supplier, as can the type of grid print used on the membrane surface. The size, colony color and medium requirements of the target organism will all influence the choice of membrane filter. The selection of the membrane used in the MF analysis technique can have a significant impact on the recoveries of colonies and therefore the accuracy of results. Incorrect results may lead to considerable consequences across different industries: a delay in batch release, costly product recall, or most importantly an impact on public safety.

cytiva

cytiva.com

CY11905-07Jul20-ED