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Membrane Filter Pore Size Selection For Microbial Testing

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INTRODUCTION

Membrane filters with a 0.45-micron pore size have long been recognized as standard for microorganism recovery, growth, and enumeration in microbial test procedures. In methods utilizing these filters, the sample is passed through the membrane filter using a filter funnel connected to a vacuum system. After filtration, organisms present will be retained on the filter surface; the membrane is then aseptically transferred to a sterile Petri dish containing appropriate growth medium. After membrane transfer, incubation is carried out at an appropriate temperature and time to facilitate the growth of organism colonies on the membrane surface. After the completion of incubation, if microorganisms are present in samples and these are culturable, visible colonies rise over the membrane; colonies are termed colony forming units (CFU) and the results are expressed as colony forming units per unit volume.

MEMBRANE FILTRATION TECHNIQUES

The membrane filtration method is an effective and reliable technique. Among the conventional culture-based microbiological methods, this technique is widely used for water samples, liquid samples, and samples which are soluble in water.

Figure 1: Microbial Filtration Technique

Advantages

Advantages of this method include the following:

- Membrane is easily transferrable from one membrane to another membrane for selection and differentiation of organisms hence it allows isolation and enumeration of discrete colonies.
- Results are more reliable than any other traditional method.
- This technique involves less sample preparation time.
- Testing can be performed large volume of samples.
- Membrane can be rinsed to remove any antimicrobial residues from the sample.
- Requires less culture media.
- Requires less glassware.

Uses

Membrane filters of various pore sizes are used for numerous applications. Pharmaceutical products such as drugs, hormones, sera, and vitamins are filtered; some may be filtered for disinfections and/or sterilization. Supplemental culture media are filtered. Testing of drinking water, purified water, Water for Injection, raw water, in-process bioburden, and pure steam requires filtration. Bacterial monitoring in the pharmaceutical, cosmetics, electronics, and food and beverage industries requires filtration. This technique allows for removal of bacteriostatic or bactericidal agents that would not be removed in pour plate, spread plate, or MPN techniques.

MEMBRANE FILTER SELECTON

Different grade and pore size filters are commercially available. Official pharmacopeia and filter manufacturers recommend use of a 0.45-micron filter for the Microbial Limit Test, pharmaceutical grade water analysis, sterility testing, and related procedures.

Enumeration, isolation, and sterility testing generally utilize 0.45-micron filters for testing. Microorganisms can be easily retained on the filter and the applied vacuum will not have impact on the viability of the recovered microorganism. Smaller sizes of microorganisms can be retained on 0.45-micron filter due to filter morphology. Membrane filters do not have single uniform pore sizes from top to bottom of the filter; see Figure 2. It is not possible for smaller (<0.45 um) microorganisms to pass thorough the filter; they are retained on the membrane.

If 0.22-micron filters and applied vacuum were used, microbial cell damage may occur causing lesser recovery during incubation. In addition, not all samples will necessarily filter through the smaller membrane. Laboratories use 0.45-micron filters for microbial enumeration testing, isolation, and sterility testing.

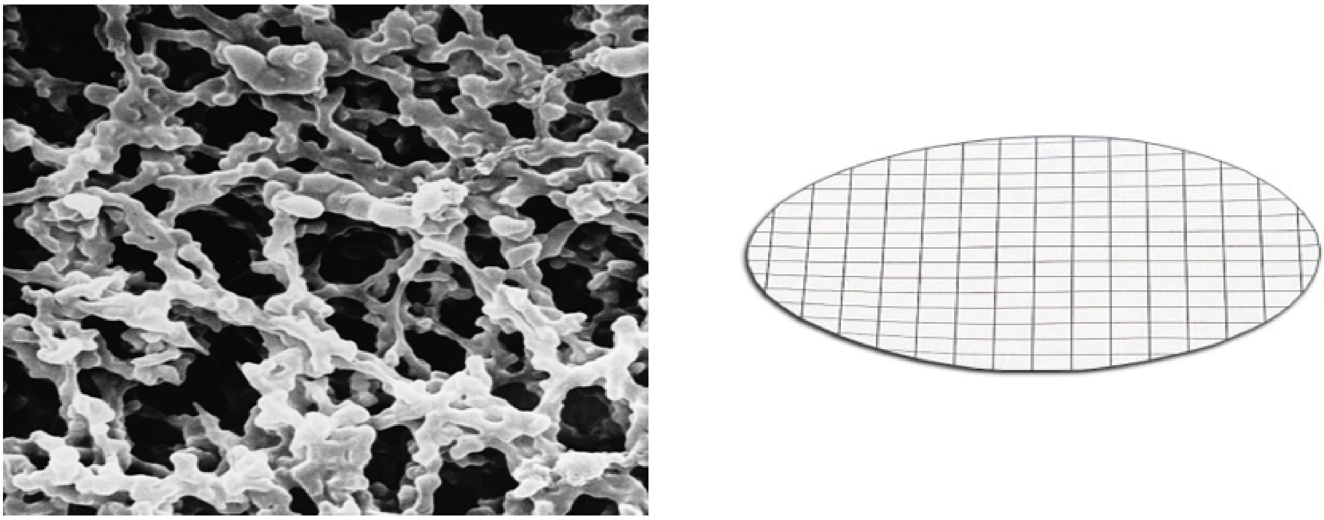

Figure 2. 0.45 µm Membrane Filter – Morphology and Gridded

RECOVERY STUDY - 0.45 um VS. 0.22 um

During filtration of disinfectants, small volume parenterals (SVP), or large volume parenterals (LVP) preparation, 0.22-micron pore size filters are used; the main purpose of these procedures is to obtain a sterile solution by removing the contamination from the solution. Whether these are alive or dead cells does not matter; what matters is that the solution must be sterile.

To demonstrate the impact of 0.45 micron and 0.22-micron filter on organism recovery, the following was conducted using three organisms. The average diameter of spherical bacteria is 0.5-2.0 µm. Rod-shaped and filamentous bacteria were length = 1-10 µm and diameter = 0.25-1.0 µm.

Escherichia coli. Rod shaped bacterium of average size 1.1 to 1.5 µm by 2.0 to 6.0 µm (Figure 3).

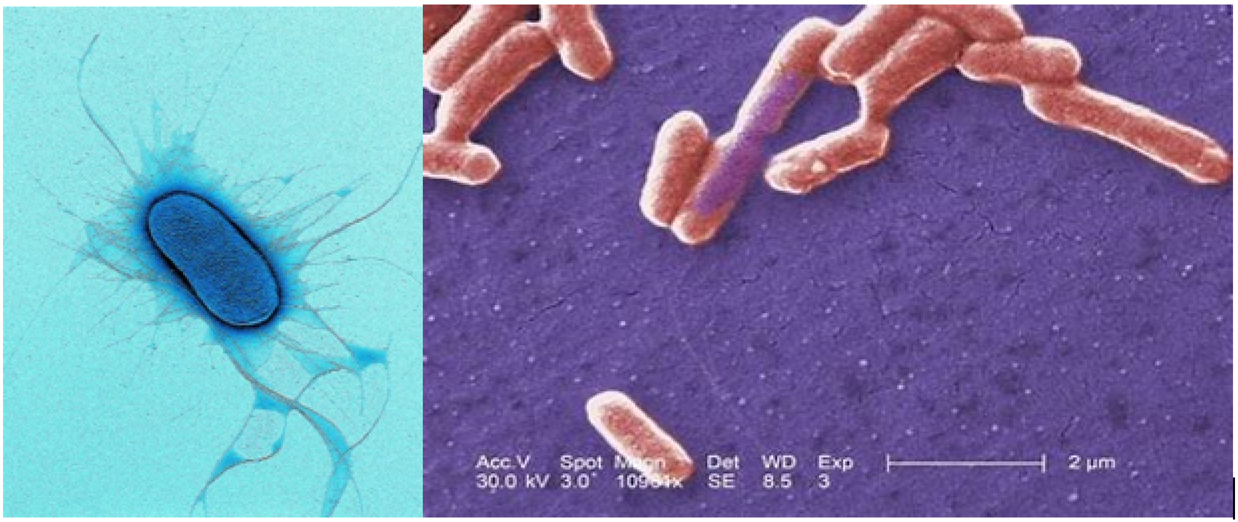

Figure 3. E. coli SEM (Scanning Electron Microscopy)

Staphylococci. Staphylococcus aureus are arranged in grape-like clusters formed by irregular cell divisions in three plains (Figure 4).

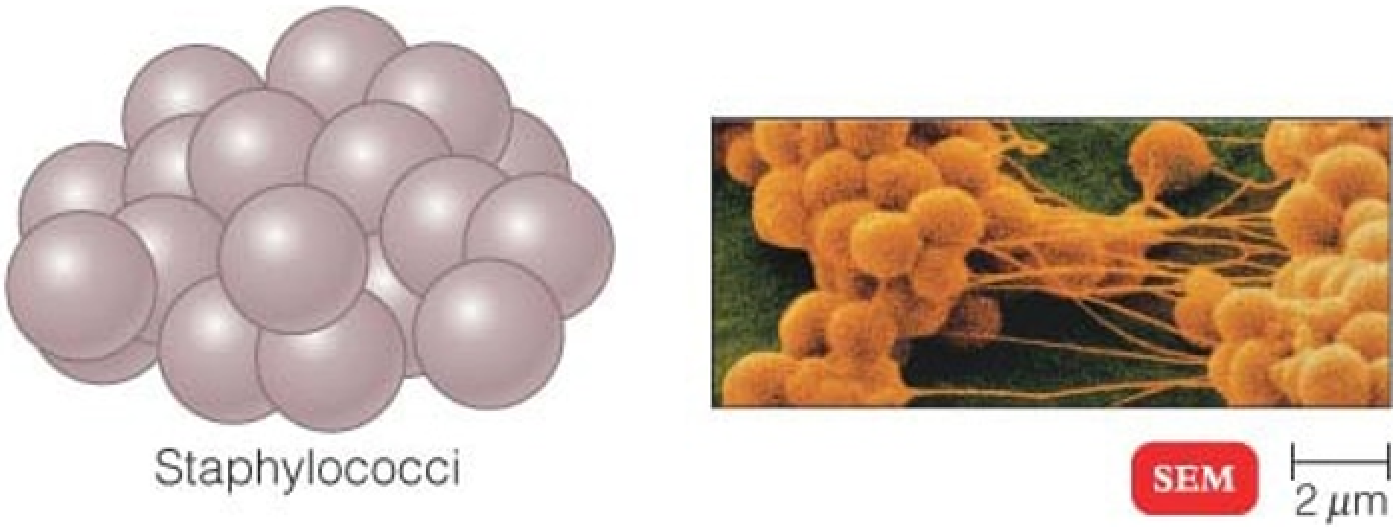

Figure 4. Staphylococci SEM

Bacilli. Rod-shaped bacterium*B. subtills* used (Figure 5).

Figure 5. Coccobacillus SEM

Procedure

Bioball® Multishot-550 (Biomerieux) was used for the study. Concentration: 0.1 ml contains 55 CFU organisms. The medium used was SCDA.

Results are presented in Table 1. Colonies on 0.45 micron and 0.2-micron filters were the same size or somewhat smaller than colonies grown on spread plates.

Table 1. Recovery Study For 0.45 Micron and 0.2-Micron Filters

	0.45 MICRON FILTER (CFU)	**0.22 MICRON FILTER (CFU)**
Escherichia coli	41,48	7,5
Staphylococcus spp.	44,52	18,27
Bacillus subtilis	50,42	21,34

Note: Because of confidentiality only microbial counts are recorded.

0.45 µm filters demonstrated acceptable recovery (? 70% vs. spread plates) for all tests. Although 0.22 µm filters gave acceptable recoveries against some organisms, their average recovery was significantly lower overall. 0.22 µm filters are used to sterilize pharmaceutical products but are not favoured for microorganism recovery in microbial testing.

CONCLUSION

This study supports the use of 0.45 µm pore size for recovery of microorganisms. Hence 0.45 µm is recommended for Microbial Limit Test, pharmaceutical grade water analysis, and sterility testing.

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