

<b>Department</b>	<b>Micro Laboratory</b>	<b>Document no</b>	<b>MICLAB – METHOD 003</b>		
<b>Title</b>	<b>Membrane Filtration Techniques</b>				
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## 1. SCOPE AND APPLICATION

This general test method sets out the procedure to be followed for estimating the number of colony forming units (cfu) using the membrane filtration technique.

This procedure is applicable only to liquids, which can be efficiently filtered without causing a build-up on the membrane (eg. Listerine, Ora-sed jel) and will be followed to test purified water, rinse water and alcohol.

## 2. REAGENTS AND MATERIALS REQUIRED

2.1 Membrane filtration assembly unit consisting of:

- 2.1.1 Manifold
- 2.1.2 Magnetic filter holder (funnel)
- 2.1.3 S/S collecting vessel and clamp
- 2.1.4 Vacuum hoses

2.2 Grid marked 0.45µm membrane filters of 47 mm to 50 mm diameter (sterile), individually wrapped. Hydrophobic edge membranes for products, hydrophilic edge for testing of water and Supor-200 47mm 0.2µm for alcohol.

2.3 A source of vacuum.

2.4 Forceps – flat tip.

2.5 Sterile graduated pipettes.

2.6 Appropriate media required for a particular product test.

2.7 Laminar flow unit.

2.8 Ear plugs (optional)

2.9 Gloves

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### 3. GENERAL TEST METHOD

**SAFETY NOTE: Do NOT use Bunsen Burner when filtering products containing alcohol.**

- 3.1 Decontaminate laminar flow unit by turning on the UV lamp for 15 minutes. Open unit and decontaminate work bench using an approved decontaminating solution.
- 3.2 Place manifold on bench.
- 3.3 Aseptically remove filter funnel from the autoclave bag by holding the top of the funnel in one hand and opening the autoclave bag at the base of the funnel with the other hand and exposing only the filter funnel neck.
- 3.4 Place filter funnel in the manifold suction base.
- 3.5 Attach the vacuum hoses to the manifold and s/s collecting vessel. Ensure that the vacuum taps on the manifold are closed (horizontal position). If the collecting vessel is not a safety vessel (ie coated in wax) place vessel into steel canister and seal lid down with clips.
- 3.6 Remove sterile forceps from packaging.  
  
Note: Ensure flat tip forceps are used, these will not puncture the membrane.
- 3.7 Hold forceps in one hand. With the other hand remove from a box a single envelope containing the filter membrane or use ezi pak dispenser.
- 3.8 Using both hands gently open envelope to expose membrane. Ensure that forceps do not touch any part of the envelope.
- 3.9 Hold envelope in one hand and remove membrane using forceps held in the other hand.
- 3.10 Lift the top of the filter funnel with one hand and place the membrane with the grid-marked side upwards onto the filter support screen.
- 3.11 Replace the top of the filter funnel.
- 3.12 Pour the required volume of the sample into the filter funnel.
- 3.13 Gently apply vacuum to draw sample through the membrane. (ie. turn the vacuum tap on the manifold to the vertical position - ON).

If membrane filtration technique is used to test the product, then the membrane and inside of the filter funnel must be rinsed with an appropriate rinse solution.

If volume to be filtered is less than 10 mls add about 50 mls of diluent to the funnel before addition of the sample to aid uniform dispersion of the bacteria over the entire surface of the membrane during filtration or alternatively, add the volume to be tested into the bottle containing sterile diluent - mix well and then

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pour the entire volume into the funnel. Some products require rinsing with Diluent and Tween to ensure that maximum filtration of the product has occurred. Refer to validation methods for the number of rinses for the product.

- 3.14 Lift the top of the filter funnel.
- 3.15 Remove membrane with sterile forceps, replace the top of the funnel. Ensure a new pair of sterile forceps are used for each transfer of the membrane.
- 3.16 Place membrane onto required agar plate by rolling the membrane, grid-marked side upwards, taking care to avoid entrapping air bubbles between the membrane and the agar surface.
  - When enrichment procedure is required, then roll the membrane into a tube using two pairs of forceps then hold the rolled membrane in one hand, pick up a bottle of media with the other hand. Unscrew the lid, pass the neck of the bottle through flame and then gently insert the membrane into the bottle.

Flame the neck of the bottle and replace lid.
- 3.17 If membrane filtration technique is used to test a product, a 100mL of rinse solution such as DIL+Tween must be filtered and placed on appropriate media.

NOTE: This will be diluent control plate.

**SAFETY NOTE: Take care when lifting and emptying collecting vessel in the sink to avoid waste splash back.**

#### 4. REFERENCE DOCUMENTS

None

#### 5. REVIEW HISTORY

Version #	Revision History
MICLAB – METHOD 003	New