

## **A SPREADFILTER IS UNLIKE ANY OTHER FILTRATION APPARATUS!**

Compared with other HGMF filters it saves a busy lab much time and labour. It is simple to use, but needs a special technique. Please read the following before using your Spreadfilter.

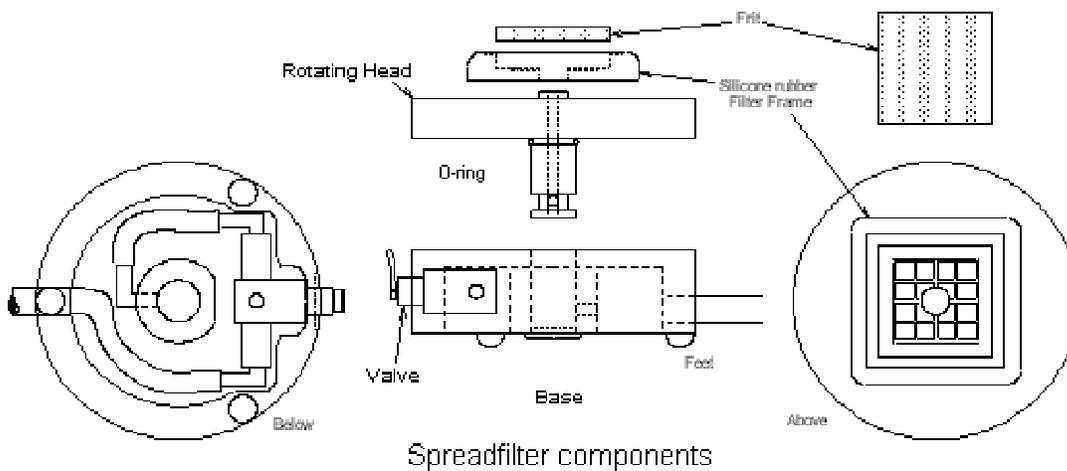
### **THE HGMF SPREADFILTER TECHNIQUE**

1. Diluent **MUST** contain 1% Tween 80.
2. Before laying an HGMF on the Spreadfilter pour a puddle of sterile diluent on the glass frit to help centre and "bed down" the HGMF. Open valve momentarily to suck down the HGMF.
3. With the pipette *vertical* pipette 2-5 ml of inoculum into the *centre* of the HGMF.
4. Hold the pipette at a low angle and "paint" the inoculum along one HGMF border. Wet about 1/3 into the border as you paint.
5. Rotate the Spreadfilter Head 90° and paint again. Rotate and paint two more times the same way, to inoculate all the HGMF surface.
6. Raise the pipette to vertical again to drain out any liquid that has re-entered it.
7. Open valve and suck inoculum through.
8. Re-use the Spreadfilter immediately if further samples are to be processed.



#### NOTES:

- i) For 1 ml inocula, pour 2-5 ml sterile diluent on the HGMF and quickly mix before spreading.
- ii) Spreadfiltering 10 ml inocula is possible but requires a *steady hand*...
- iii) Spread inocula *quickly* to minimize bacterial attachment and uneven inoculation.



### HOW DOES A SPREADFILTER DIFFER FROM OTHER FILTERS?

	What is different	Why?
1	No funnel is needed when filtering volumes less than 10 ml.	The hydrophobic border of the HGMF acts as a wall.
2	It does not need sterilizing between samples.	<ul style="list-style-type: none"> <li>- there is no funnel to get contaminated;</li> <li>- the HGMF keeps sample bacteria from the fritted glass;</li> <li>- the occasional contaminant <i>under</i> the HGMF will not affect what grows on top.</li> </ul>

### APPLICATIONS

	When to use the Spreadfilter	When NOT to use it
1	Routine work on samples with "normal" contamination levels	Sterility testing or other critical work..
2	On samples of low microbial hazard.	If severe microbial hazards may be encountered.

### WHAT YOU NEED

	Prerequisite	Why?
1	Horizontal, steady bench surface.	A uniform liquid depth leads to uniform inoculation.
2	Membrane filter forceps.	
3	Sterile diluent containing 1% Tween 80 (eg, in 100 ml bottles).	<ul style="list-style-type: none"> <li>- To prewet and seat the HGMF;</li> <li>- for expanding small sample volumes.</li> </ul>

## HOW TO USE THE SPREADFILTER (see pictures on page 1)

	What to do	Why
1	Liquids must contain 1% Tween 80	Otherwise they will not spread on the HGMF
2	Pour a puddle of 2-3 ml of sterile Tween diluent onto the glass frit before placing the HGMF.	<ul style="list-style-type: none"> <li>- the liquid helps you centre the HGMF;</li> <li>- a wet HGMF minimizes sample penetration, improving uniformity of CFU distribution.</li> </ul>
3	Open the valve <i>slightly</i> to suck the HGMF into contact with the glass frit.	Opening it excessively may leave a residual vacuum that pulls the sample aliquot through before it can be spread.
4	<i>Optimal volume</i> to filter is 2-5 ml. To filter 1 ml or less, place 2-5 ml diluent on the HGMF, add the sample aliquot and quickly mix it in before spreading.	<ul style="list-style-type: none"> <li>- Samples less than 1 ml may sink into the HGMF before they can be spread. Samples of 10 ml require a very steady hand;</li> <li>- (Note: a sample that is diluted on the HGMF this way need not contain Tween 80 provided the diluent does).</li> </ul>
5	With the pipette <i>vertical</i> add liquid to the <i>centre</i> of the HGMF.	If the pipette is on an angle, liquid may squirt over the side of the HGMF.
	<b><i>Do steps 6-10 quickly:</i></b>	Bacterial attachment to the HGMF surface may cause uneven inoculation.
6	With the pipette almost horizontal, spread liquid along one HGMF border. Spread onto about 1/3 of the border.	<ul style="list-style-type: none"> <li>- the goal is to have a uniform liquid depth over the entire HGMF;</li> <li>- running liquid on the border prevents the meniscus depleting CFU concentration near the border.</li> </ul>
7	Rotate the Spreadfilter 90°, spread the next border, and so on to flood all the HGMF.	
8	Raise the pipette to <i>vertical</i> to drain again.	Liquid will have re-entered the horizontal pipette.
9	Open the valve to suck liquid through.	
10	Remove and plate the HGMF as soon as the filtrant has run through	Exposed bacteria die off very quickly on membrane filters.
11	<i>Re-use the Spreadfilter immediately without sterilizing it.</i>	See explanation above.
12	<i>End of day:</i> flush with 70% ethanol, dilute formaldehyde, or other disinfectant.	Replace sample residue that may serve as growth substrate by microbial growth inhibitor.
13	<i>If sterilization is needed:</i>	See instructions below.

## ASSEMBLY AND DISASSEMBLY OF THE SPREADFILTER

1. The glass Frit lies loose within the silicone rubber Frame. The Frame itself simply lays on the Rotating Head, centralised by the Nipple, and sealing for vacuum by its flexibility.
2. To disassemble, simply lift the Frame from the Head, then invert it and flex it slightly to allow the Frit to be eased out. Pull the Rotating Head from the Spreadfilter Base.
3. To reassemble, insert the Rotating Head in the Base. Lay the Frame over the Nipple and flex it in a circular movement until it drops snugly onto the Head.

## ROUTINE CLEANING AND LEVELS OF CONTAMINATION

- 1 Simply flushing the Spreadfilter with 70% ethanol 2-3 times/h, and at the end of the day, prevents contamination problems when working with routine food or environmental samples. This is because the HGMF filters out microbes from the sample and prevents them reaching the glass frit. Although the frit may transfer the occasional microbe to the underside of the HGMF, contact between the HGMF and the agar is strong enough to prevent contaminants growing significantly under the HGMF and perturbing the normal growth of target colonies on its surface.
- 2 In routine use it is common to observe a proportion of plates with small amounts of growth on the agar along the border of the HGMF. *These are not usually cause for concern.*
- 3 The only known serious problem is occasional infection by a swarming *Bacillus* spp., which produces a massive crenated lawn on the agar, and also grows into the HGMF. *(This organism causes similar problems with HGMFs inoculated by the ISO-GRID filtration apparatus. which is autoclaved for every use).* The contaminant actually enters *via ethanol* used for flaming forceps or flushing. If this problem occurs, autoclave or dry flame all apparatus used for HGMFs, and procure fresh ethanol.

## STERILIZING

Autoclaving the Spreadfilter Base is not advised. If the Rotating Head is autoclaved, lay it spindle uppermost so as to prevent warping.

For normal sterilization the glass Frit and silicone rubber Frame can be lifted off and autoclaved alone. **CARE: the Frit is easily damaged. Frits may soften slightly after autoclaving or after prolonged use - a spare Frit is included, replacements may be ordered at nominal cost.**

## SUGGESTIONS IN CASE YOU EXPERIENCE UNUSUALLY SLOW FILTRATION

Membrane filtration of food suspensions is generally straightforward. Factors to consider in order filter rapidly and reliably through ISO-GRID HGMF™ Hydrophobic Grid Membrane Filters are dealt with in a companion document (**Filtering Food Suspensions**). Here, we deal with problems specific to the use of the Spreadfilter.

Your Spreadfilter will give relatively trouble-free service but keep in mind that, simply because it is such an undemanding device, it tends to be used hundreds of times more between service operations than does any competitive filtration apparatus. The daily combination of bacteria, food suspensions (and, possibly, hurried "end of day" care), may eventually lead to impaired performance. Reduced filtration rates through HGMFs after long periods of good performance may result from several causes, listed here in order of decreasing probability.

### **1. Blocked passages in the Base Unit**

The most common cause of slow filtration rate through a Spreadfilter is a build-up of organic material in the Base Unit. Formation of mucin from even low concentrations of residual organic matter can eventually block the passages of a Base Unit. With the small air leakage that usually occurs past the HGMF the result can be a substantial reduction in vacuum at the HGMF, and slow filtration.

*The passages in the Base Unit are unblocked easily, either by dismantling and flushing the individual pieces by water pressure, or by using compressed air in the reverse direction (caution!).*

If a blocked Base Unit turns out to be the cause of the difficulty, *flushing with extra distilled water before* running the customary 70% ethanol through should help - we would suggest at least 100 ml. Open the Valve and pour water on the Frit rapidly enough to keep it flooded. Test for blockage by removing the Spreadfilter Head, filling the spindle tube with water and opening the Valve (with vacuum on) - the water should disappear very quickly.

### **2. Low porosity HGMF batch**

Occasional lots of ISO-GRID HGMFs filter slowly, though they behave well otherwise. The lab of A.N. Sharpe (Health Canada) used to order samples of several lot#s, testing filtration rate, uniformity, and barrier integrity. There *were* differences and they chose the best lot; however, the manufacturer (Neogen Inc) may only be happy to go along with this if you order HGMFs by the 1,000's...

### **3. Blocked Glass Frit**

Organic matter can build up in any glass Frit over time. If this is suspected, simply replace the Frit.