

FAQ's and USEFUL INFO RE SPREADFILTERS AND REPLICATORS, ETC

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(Celite and filter-aids; glycerine; green stain from cow faeces; incomplete filling of grid-cells; roughness of the HGMF print; contaminants appear on replicated HGMFs;)

CELITE - (filtration aid)

We did some work several years ago to improve the membrane filterability of chicken rinses.

The (paint shaker) rinse of a "roaster" in 100 ml diluent only filters reliably about 10 ml through an HGMF. The goal was to filter it all as part of a *Salmonella* and *Campylobacter*-enumeration project. We tried many "filter-aids" with minimal success, but Celite (diatomaceous earth) was best.

By adding Celite to the rinse, shaking, and allowing to settle for a while, the supernatant filtered dramatically better and >90% of the bacteria remained in suspension. Alternatively, with the right amount of Celite, the whole could be filtered, the Celite on the HGMF providing a cosy growth environment without bridging the grid-cells. I don't have access to the lab notebook, but from memory:

- it was essential to use "crude" Celite. "Pure" Celite (which is calcined at >500C to remove protein and other "impurities") mopped up everything in reach, including bacteria, and supernatant counts were almost zero.

- about 5% Celite by weight was more than enough – however, this is very bulky and thoroughly cakes the HGMF if the whole rinse is filtered.

- there is a wide particle size range in Celite, so settling produces a continuum of precipitate – longer (20 min) settling and pipetting-off less gave better and better filtration with less cake on the HGMF, but a smaller proportion of the whole to filter.

GLYCERINE

If glycerine is used as an aid to freeze-storage of HGMF cultures, it may get used during a spreadfiltering step. I don't think glycerine should affect spreadfiltering - certainly at low concentrations. If one is filtering, say, 20% glycerine I would expect perhaps a small "loss" of count due to some of the suspension remaining across grid lines - after plating on agar the glycerine would slowly absorb water and creep off of the line, but some of the cells in it might by that time have adsorbed to the line ink and would not follow the liquid. Filtering a small volume of regular PT diluent after spreadfiltering might prevent such an occurrence.

GREEN STAINS FROM BOVINE FAECES

The HGMF may be stained green when filtering bovine faeces and this may interfere with recognition of colony colours. The "chlorophyll stain" is actually green particles, otherwise it would filter through the HGMF and be completely removable by a 2nd rinse. Ability to solve it easily depends on what size the particles are, and how strongly target cells are attached to them.

The particles are likely to pass through FiltaTips (which are quite coarse filters). Possible solutions to green stains are:

1. Allowing a settling period - if the green settles and the target doesn't, great!
2. Shaking with a small amount (say, 1% or less) of crude (not purified) Celite (see above). Bentonite clay is another possibility. Normally, I would recommend allowing the filter-aid to settle and testing the count in the supernate - but see #4 below...
3. The Neogen/QA Lifesciences filtration apparatus has a (claimed) 5 micron filter - put in because some of their first customers (1980) complained that chili powder, cocoa, and turmeric made grids hard to count - QA did tests to show that much of the colour was removed without effect on the count. If you have a Spreadfilter I hate to suggest buying something else - but it presents one possibility - however, you then has to live with cleaning and sterilizing each QA apparatus every time you use it - so you would need several.
4. My final suggestion also might solve a problem of growths not filling the grid-cell - and can be used at any dilution of cells:- EITHER - do not let the Celite (maybe in higher than 1% concentration) settle - filter it down onto the HGMF - you will have to adjust the concentration/volume so that you get a little pile/mat of Celite on the grid-cell but not so much that the grid-line itself gets obliterated; or - after filtering the suspension down firmly, gently add a small amount of Celite suspension and filter on top of it - again, not obliterating the grid line - diluting it or covering it over with white powder may reduce or eliminate the green stain.

INCOMPLETE FILLING OF GRID CELLS

Often colonies do not grow sufficiently vigorously to fill grid-cells completely during the incubation period. The problem may be due to injury, or reluctance to grow, when cells are relatively exposed to the air as they are on the surface of a membrane filter. Addition of small amounts of Celite or other powders (such as cellulose powder) to suspensions can be a great help - having a nice moist mat to grow in allowed both *Listeria monocytogenes* and *Campylobacter jejuni* colonies (that otherwise grew poorly and were unable to completely fill the grid-cells) to grow much more vigorously - the Celite particles are very much larger than the bacteria and the voids between particles remain full of water, so it's almost like the bacteria have a broth to swim around in.

HGMF REPLICATES ARE "ONE-SIDED" OR LOOK LIKE MOIRE PATTERNS

A badly aligned Inoculator can come down with many of the teeth touching grid-lines or intersections, giving the daughter HGMF a "Moiré pattern" inoculation that leaves much of the HGMF bare. Refer to the Replicator manual - you may need to check alignment with an ink pad.

SLOW FILTRATION THROUGH HGMFs

This topic is covered in the Spreadfilter manual. Reduced filtration rate through HGMFs may have several causes:

1. The most common cause of slow filtration rate through Spreadfilters is a build-up of organic material *in the base unit*. Bacterial growth easily produces mucins from even the lowest concentrations of residual organic matter. I have known units block up completely that way. Coupled with a small amount of air leakage this can result in a very substantial reduction in vacuum at the HGMF, and thus a very slow filtration. The passages in the Base Unit are unblocked easily, either by dismantling and flushing by water pressure, or by using compressed air in the reverse direction (caution!). If this turns out to be the cause of the problem, flushing with extra DI water before running EtOH through should help - use at least 250 ml. 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. The water should also run through with no vacuum on, though surface tension and bubbles may prevent the flow starting.
2. Check that reduced filtration rate isn't being caused by or exacerbated by low vacuum as a result of air leakage - if you have a clear PVC tube anywhere in the vacuum line you'll see excessive foam/bubbles going through. I mean *excessive* - there will always be *some* air. The ability of the Silicone Rubber Insert to seal nicely in the Head is tested before shipping. If you aren't getting a good seal a little Vaseline wouldn't hurt - I would use this rather than silicone vacuum grease which eventually spreads everywhere and is very difficult to remove (did you know it has been shown to spread out until it eventually forms a molecular monolayer throughout every lab that uses it?). Check that the Silicone Rubber Insert is seating well at the corners (sometimes it needs to be just pressed down there). I would also check the seal by laying a smooth rigid piece of plastic on the rubber, then turning on the vacuum.
3. I have had the odd comment about the Silicone Rubber of the Spreadfilter rubber going "brittle and particulate". My own experience has been that, on excessive autoclaving the silicone rubber tends to become softer and a bit sticky ("tacky"). In either case, replace the Silicone/Frit unit - the new design is inexpensive.
4. There have been batches of ISO-GRID HGMFs that were inherently low porosity. They behaved OK otherwise, but were slow filterers. At HPB we used to order samples from several lot#s and test for filtration rate, uniformity, and barrier integrity. There were differences and we chose the best lot - of course, HPB used them by the 1,000's, so QA Lifesciences was happy to go along with it.
5. Organic matter *could* have slowly built up in the sintered glass, but this is less likely. Sintered glass was introduced into Spreadfilters several years ago. Before that we used a silicone carbide/epoxy mix that (over several years) eventually softened; however, I never saw a SiC one block up, to the extent of reducing filtration rate. If you find this really to be the case, it could be mucin, which is not that easy to remove chemically. Soaking in a mixture or succession of enzymes (hemicellulase, trypsin, amylase) might help. The best answer is to replace the Insert.
6. Older models that have a molded Sintered Glass/Silicone Rubber Insert need to be handled carefully otherwise the rubber may tear at the glass. Provided there is no actual loss of material

this should not affect filtration - once the wet HGMF is under vacuum you should still get a vacuum circuit; however, a little Vaseline around the perimeter certainly might help. There is no practical way you can re-attach the silicone rubber - silicone adhesive will probably make your seal problem worse. Bearing in mind the short lifetimes of more conventional types of membrane filtration apparatus one should not regard an item such as the Silicone Rubber Insert as having an indefinite life. The best solution would be to trade up to a newer Head that takes the 2-piece Silicone/Frit.

CONTAMINANTS APPEAR ON COPIED HGMFs ALTHOUGH THE MASTER HGMF APPEARED "CLEAN"

A typical occurrence is that a master HGMF of *Escherichia coli* is prepared from faeces on a selective medium such as Violet Red Bile agar. The master HGMF appears to contain pure *E.coli*. However, daughter (copy) HGMFs prepared on agars such as Mueller-Hinton base plus antibiotics, show signs of contaminants after incubation.

Contaminants appear because, while moderately selective, the initial agar (VRB here) does not kill all non-*E. coli* in the faecal inoculum. Because there may be very large numbers of non-target cells in the faeces, it is very likely that some of these will be transferred during when the HGMF is replicated, and they will grow on the less selective MH agar. This is a difficult problem, because increasing the selectivity of the MH agar may invalidate test results. Possible remedies are:

1. Avoid any non-selective step such as a short resuscitation on TSA.
2. Make a second copy of the master HGMF onto VRBA before proceeding with replication for profiling - this will introduce a second "dilution" step that may eliminate or minimize carry-through of contaminants.
3. Or, after they have grown, recopy the MH/antibiotic HGMFs onto fresh VRBA HGMFs, so that the *E.coli* become more visible.
4. A further (suggestion only) might be to add crystal violet and neutral red (or even just the red) to the MH agar to make it easier to see the *E. coli*, even if contaminants do grow. Possible problems: - I'm not sure whether the pH change would be sufficient to promote colony colouring, and the dyes would have some effect on selectivity - but it may be worth a try.