

## Sample Preparation Guidelines

Here is a summary of some things that can be done prior to assay, to help prevent well blockages when using sera with 96 well filter plates.

- Always spin the samples down to begin with (eg.7000rpm for 5 minutes). This will remove any large debris from the fluid. When pipetting the samples always use fresh tips and take the fluid from near the top or towards the centre of the vessel, not the bottom.
- For particularly viscous samples pre-filtration steps can be done. These will be explained below.
- Put the sample volume (+5%) required for the assay in each well on a filter plate. Place another ordinary plate below the filter plate. Spin these 2 plates in a large centrifuge (the spin speeds and any adapters required can be obtained from the relevant plate manufacturer). The sera should pass through the filter and be ready to transfer to a fresh filter plate for assay.
- Another way to pre-filter is to use the vacuum manifold. Add the relevant sample to each well on a filter plate. Put this on the manifold with a normal plate underneath the filter plate, so the wells are directly in line. Vacuum the filter plate at a low pressure (once again this will differ for each type of manifold so the manufacturer will advice further). Once the sample has gone through the filter plate into the normal plate, the samples can be transferred to a fresh filter plate for assay.
- For large volumes of sera, filtration tubes can be used. They are like plastic test tubes with a filter membrane in the middle. The chamber at the top holds the sample and when centrifuged, the sample is pushed through the filter. This would allow filtration of a larger volume of sera in one process ready for assay. An example of a company that produce these kinds of filters is [www.pall.com/gelman](http://www.pall.com/gelman)
- The final advice is to try different filter plates from different manufacturers. For example, Millipore, Corning and Nunc all have a range of filter plates and some may work better than others. There are other companies that also sell filter plates for specific applications so an Internet search, can sometimes be a good way to investigate products available for specialist work.

## Vacuum Manifold Guidelines

If the filter plate does block during a wash the best course of action is:

- Make sure that a sealed vacuum is present. This can be done by placing a plate sealer over any empty wells, or alternatively using the palm of your hand to cover any empty wells.
- Use a pipette to gently mix the sample in the blocked well. Slowly fill and empty the tip with fluid to help move any debris from the bottom of the well. It is advisable to decrease the pump pressure if possible during this sample-mixing step.