Sterility testing of difficult-to-filter and growth-inhibiting samples.

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An important question for many companies in the pharmaceutical industry is whether to use membrane filtration or direct inoculation of liquid culture when developing new sterility testing methods. USP <71> recommends membrane filtration as the preferred method for sterility testing whenever the nature of the product permits. Filtration methods have the advantage of allowing large sample volumes to be tested for meeting USP requirements and recommendations. Also, for products with antimicrobial activity, filtration allows rinsing and thus the removal of the inhibitory characteristics of the product.

While membrane filtration is often straightforward, some sample types can be challenging. These include difficult-to-filter viscous oils, water-in-oil emulsions and fatty base ointments as well as samples containing substances that inhibit microbial growth. High-density cell cultures can also prove problematic. However, membrane filtration can be made to work for most such products perfectly well.

IPM and PVDF membranes can help with creams and ointments

To improve filterability, viscous products are normally diluted in a sterile solvent. In many cases the USP's Fluids A, D, or K can be used as diluents. Creams and ointments, however, often require stronger solvents such as isopropyl myristate (IPM), a fatty acid ester that is compatible with the low-adsorption PVDF membranes of solvent-resistant canisters for sterility testing according to USP. Another way to facilitate filtration is to raise the temperature of the diluent. It should not exceed 40 °C, although under exceptional and justifiable c ircumstances, U SP <71> allows temperatures of up to 44 °C.

A different kind of challenge is growth inhibition caused by the product, which can be an issue when the product is an antibiotic. USP <71> gives clear guidance on how to perform sterility testing of antibiotics. For example, the membrane must be prewetted and rinsed no more than five times (unless justified in certain cases) with 100 mL of rinsing fluid before the culture medium is added. In addition, the test volume must be kept to an acceptable minimum. It is recommended to pool an appropriate number of samples in a single bottle and filter the contents using canisters with a PVDF membrane. This minimizes the contact time of the product on the membrane and, like prewetting, limits adsorption.

Lysis solution can help with high density cell based products

The difficulties posed by mammalian cell cultures tend to increase with the density of the cells. However,

special sterile lysis solutions containing detergents can help in many such cases. Lysis protocols have been developed that can clear the way for subsequent membrane filtration.

All procedures for challenging samples are determined during method development, which is the initial step for each challenging sample type. It is often outsourced because, due to the nature of the production process and the need to release finished products in a timely manner, many companies find it difficult to allocate resources to method development. Another reason can be a lack of in-house know-how and experience in optimizing the filterability of challenging samples.

Examples of filterability projects

A past project to achieve solubility and filterability of an oily pharmaceutical product exemplifies the intricacies of method development. Initially, a 5 mL sample was diluted in Fluid K, but during subsequent vortexing the sample gelled. Next, a 1 mL sample was diluted in 20 mL Fluid K and vortexed vigorously, after which more Fluid K was added up to 100 mL. However, only a small volume could be filtered before the plug needed to be removed to ease the pressure in the sterile filtration canister. In the scheme that eventually proved successful, 1 mL of the oily sample was diluted in 20 mL of saline with 0.5% polysorbate 80 and vortexed before Fluid K was added up to 100 mL. The entire sample content in 100 mL could be filtered through a PVDF membrane.

In a different project, a sample of viscous antibiotic ointment was successfully filtered using warm IPM. A sample portion was diluted in 100 mL of warm (44 °C) IPM according to USP recommendations. The membrane was prewetted with 50 mL of warm IPM before the diluted solution was filtered. The canister was then rinsed three times with 100 mL of Fluid K. Each time, rinsing was stopped after half the 100 mL of Fluid K had been filtered, and the canister swirled to rinse the sides. In accordance with best practices, a final rinse of the membrane using 100 mL of Fluid A was performed before adding the medium.

If you have filterability challenges and would like to know more about our method development services and products, contact us at PharmaQuestions@MilliporeSigma.com