Prevention Instead of Decontamination

From Filtration to Incubation in Microbiological Quality Control

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The highest possible quality of a final product in compliance with requirements and regulations, can be attained only if quality assurance is not merely limited to final product testing. Rather, the entire manufacturing process, besides incoming quality control of the raw materials used, needs to be continuously monitored.

In the pharmaceutical industry, risk analysis of individual manufacturing steps is performed and the results of this analysis are used to define in-process quality control tests. Such QC tests permit timely detection of inconsistencies or nonconforming items and, in particular, increases in the bioburden as they occur in manufacturing so that corrective actions can be promptly initiated. Even though the risk of contamination has been considerably reduced by GMP-compliant production, decontamination and sterilization of the final products, as well as by strict hygiene standards, quality control of the final product continues to be of prime importance.

Microbial Enumeration

Quantitative analysis of microorganisms involves counting the colony-forming units (CFU), hence the term "microbial enumeration." This number can be expressed either the total viable number of CFUs in general or of certain product-relevant species of microorganisms. This is why microbial enumeration tests are performed on various products from different sectors, including the pharmaceutical, beverage and wastewater industries, to ensure that defined limits are not exceeded. The accuracy and reliability of microbial limit test results are essential as they serve as the basis for the release of products, whether potable water or pharmaceuticals. The impact of undetected pathogens can be potentially devastating on the health of consumers.

Membrane Filtration

For microbial enumeration, membrane filtration continues to be the method of choice for reliable quantification of microorganisms in liquid samples. The principle of this method is based on the concentration of organisms – which are filtered out of relatively large sample volumes – on the surface of a membrane filter and their subsequent cultivation by incubating the filter with the retained microbes on a culture medium.

Unlike direct incubation of a sample, membrane filtration offers the advantage that large sample volumes can be tested without individual microorganisms going undetected. In addition, inhibitors, such as antibiotics or preservatives, can be removed by rinsing the membrane with buffer so that microbial growth is not suppressed.

Fig. 1: A separately removable interior lid provides easy access to select colonies after incubation for further analysis.

Microbiological Tests in the Pharmaceutical Industry

From a microbiological point of view, pharmaceuticals can be subdivided into two categories: non-sterile and sterile products. For both categories, the potential risk resulting from microorganisms and their toxins on patients' health must be eliminated or at least mitigated. At the same time, the quality and effectiveness of such pharmaceuticals must be retained.

Products defined as sterile, such as eye drops, physiological saline, antibiotics, etc., need to be tested for sterility (USP Chapter 71 and EP, Chapter 2.6.1) in order to be verified as such. Unlike sterile products, non-sterile final products are tested for their number of viable microbes according to the microbial limit test (USP Chapter 61 and EP Chapter 2.6.12). Furthermore, in the pharmaceutical industry, microbiological in-process quality control is carried out on raw materials, mostly water, as well as bioburden analysis during manufacture.

Critical Steps in Microbial Enumeration

The classic equipment setup for performing membrane filtration consists of a vacuum pump, a multi-branch vacuum manifold, membrane filters, reusable funnel-type filter holders or single-use filtration units, culture media and tweezers.

In this method, the filter support of a reusable filter holder is sterilized by flaming, and a membrane filter is subsequently placed on this support. Then the funnel is attached to the support and a sample is poured into it. At the end of filtration, tweezers are used to remove the membrane filter and transfer it to an agar culture medium. The culture medium is incubated for a defined time at a predetermined temperature inside an incubator. At the end of incubation, evaluation is done by enumerating the individual colony-forming units (CFUs) and comparing their count with the permissible microbial limits for each particular sample.

Flaming or disinfecting the filter support poses an added risk of contamination due to the inherent inaccuracy in performing these sterilization procedures. In particular, maintaining the required time of contact with the flame or disinfectant, the choice of disinfectant (not just a bactericide, but a sporicide) and regular changing of the disinfectant are all critical factors in determining whether sterilization is 100% effective. Besides representing a health hazard for lab personnel, flaming also poses the risk that not all areas contaminated by microbes are exposed to the hot-test point of the flame long enough in order to kill off these organisms.

Minimization of Secondary Contamination

A single-use filter unit does not require any decontamination, provided that a single-use filter base is used. As a result, the only critical step that remains is transferring the membrane filter to an agar medium, which increases the risk of secondary contamination and can lead to false-positive results. The reason lies in the use of tweezers to transfer the membrane. Although these tweezers are also flamed, i.e., sterilized, they can potentially carry over exogenous microbes when used to grasp the membrane.

The products of the Sartorius Microsart family increase the safety and efficiency of microbiological quality control by eliminating the need for disinfection or flaming of the filter support, as well as for using tweezers to transfer a membrane to a culture medium.

This family comprises of single-use filtration units, Microsart @filter, and agar media dishes, Microsart @media. Microsart @filter is a sterile, ready-to-use combination of a funnel, a filter base and a gridded membrane filter. This filter unit is simply connected to a stainless steel multi-branch manifold in order to directly filter a sample. Afterwards, the funnel is exceptionally easy to remove, thanks to its click-fit closure, from the manifold. This filtration unit eliminates the critical step of decontaminating the stainless steel base of a reusable filter holder.

Microsart @media agar media dishes are used for microbial limit testing. They are pre-filled with different types of agar media, sterile-packaged and, together with the single-use Microsart @filter, are ready to use immediately. These time-saving media dishes feature an innovative, active lid that permits touch-free transfer of a membrane onto agar, without using any tweezers. This active lid effortlessly lifts the membrane filter from the base of the filtration unit so the filter can be safely transferred onto the pre-filled agar dish. Once the medium dish is closed, the membrane is ready to be incubate.

Solution for Safe Membrane Transfer

The combination of agar media dishes and filtration units represents a brand-new membrane transfer and agar concept. During development, both products were tailored for each other so the active lid of the media dishes is designed to fit perfectly onto the filter unit base. As just a few steps are all it takes to proceed from sampling to incubation, this new single-use system of agar media dishes and filtration units accelerates workflows, making them cost-efficient. At the same time, the touch-free membrane transfer enables even more reliable results to be obtained, while reducing secondary contamination to an absolute minimum.

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Fig. 2: The active lid of the Microsart @media pre-filled agar dish enables a touch-free transfer of the membrane to the agar medium.