Overcoming the manufacturing bottleneck caused by bioburden testing

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To ensure that a pharmaceutical product is safe for patients, it is mandatory to determine its bioburden in a tightly regulated quality control examination. Bioburden testing (or microbial limit testing) quantifies the number of aerobically growing bacteria, yeasts, and molds on surfaces or in solutions of, for example, raw materials, process intermediates and final products. While essential from a product safety perspective, bioburden testing has proved to be a bottleneck in operational timelines, with manufacturers having to wait up to five days for the results of the compendial method test. Over this period, the next processing steps can often not commence or the final product not released to the market, while storage costs mount and patients quite possibly have to wait. Hence there is a palpable desire to reduce the time-to-result.

Why bioburden testing takes so long

Bioburden must be determined in both sterile and non-sterile manufacturing processes. Some products, such as injectables, must be sterile. For non-sterile pharmaceuticals, from skin lotions to conventional tablets, there are certain thresholds that must not be exceeded. These limits vary and depend on the product and its intended use, as well as on the microorganisms in question. The EP chapter describing the quantitative microbial examination of non-sterile products is 2.6.12. It lists the microorganisms to be used for method and culture validation, representing Gram-positive and Gram-negative bacteria, yeasts, and molds. However, these do not necessarily reflect the on-site situation, so it is often seen as a plus by the regulatory body to look for and validate against in-house strains that are more likely to be found on the product.

The EP 2.6.12 examination is basically a well-controlled aerobic plate count assay that includes complete neutralization of antimicrobial activity and a recovery analysis. Of the culture methods described in EP 2.6.12, membrane filtration has evolved as a method of choice because it is robust, economical, and easy to use. However, even this method involves lengthy incubation times of the agar plates because some contaminant species take a long time to grow into colonies that are visible to the eye and countable.

A way to speed up bioburden testing

When performing the compendial filtration test, it is possible to complement it with a simultaneous rapid test that uses the identical sample to generate much earlier results while allowing the compendial test to continue. Merck has implemented this by merging the procedures of two bioburden testing methods, a compendial one and a rapid one. The compendial method filtration system is the Milliflex Oasis® system. Its pump filters the sample through a funnel containing a filter membrane, a process that captures the microorganisms while removing any growth inhibitors. The membrane is subsequently transferred touch-free onto a media plate, which is then incubated until colonies can be seen and counted.

The Milliflex® Quantum rapid detection system is designed in a way that it uses the compendial method's membrane without impacting the integrity of that test. An enzymatic substrate is added to the membrane to selectively stain all living organisms on it with a fluorescent marker that allows detection at a much earlier stage of colony formation, typically several days. This rapid method does not destroy the microbial cells, so it is possible to place the membrane back onto agar, complete the routine compendial method test, and then, if necessary, identify the contaminants using any ID test, whether it be based on a biochemical, morphological, or nucleic acid method. Pattern comparisons show that when contaminants are detected, their configuration on the membrane of the rapid test after 24 hours is identical to that on the agar plate of the compendial method test after three days.

Valuable time gained when there is an issue

What if the result of the bioburden test exceeds the limit? A corrective and preventive action (CAPA) plan would need to be set up and the issue subsequently resolved. This can take some time, during which the production line and deliveries to the market could come to a standstill. Every day lost is costly. The material cultured in the course of the continued compendial bioburden test can be used for identification, which should eventually help point to the root cause of the contamination. Rapid bioburden testing, however, can provide an early indication that there is an issue to deal with, gaining time to gather initial information and manage resources and thus helping to speed up the investigation. This adds to the benefits yielded in the typical cases of the result falling within the bioburden test's limit, i.e. earlier product release and lower storage costs.

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