Rapid Microbiology Systems

Microbiological testing is ubiquitous throughout the parenteral industry—water system monitoring, environmental monitoring, preservative challenge testing, bioburden testing, and sterility testing, to name a few. However, we still rely on the same technology that Louis Pasteur and his colleagues used in the 19th century; that is, incubating samples with one or more sterile growth media for a period of time from 5 days up to perhaps 2 weeks, and observing the sample for evidence of microbial growth. These methods are limited by slow microbial growth rates, unintended selectivity of media, and the inherent variability of microorganisms in their response to culture conditions.

Rapid microbiological methods comprise several different technology platforms that offer significantly reduced turnaround time (hours instead of days), opening the possibility of process microbiological testing in real time. They offer increased sensitivity, accuracy, precision, and reproducibility. Some of these methods do not require microbial growth, thus eliminating the need for incubation. Some allow detection of single cells, and many detect stressed organisms that are not detected by conventional methods. Some of these technologies are automated, miniaturized, and offer high-throughput processing. These technology platforms are categorized in Table 23-4 (20).

The adenosine triphosphate (ATP) bioluminescence and fluorescent labeling methods have been studied extensively for application in the sterile products industry. The basic principle of the ATP bioluminescence method is summarized in Figure 23-8. Basically, if the test sample contains microorganisms, their growth will release ATP from cells and the generated ATP will react with the luciferin substrate/luciferase enzyme from the firefly *Photinus pyralis* to generate light that can be measured with a highly sensitive and accurate luminometer. One commercial example using this method is the PallChek™ Rapid Microbiology System with a sensitivity of 1 CFU. Less than 1 minute is required for immediate quantification of contaminants while product release testing requires less than 24 hours.

The fluorescent labeling method (ChemScan® RDI by Chemunex, Paris) uses a filter to trap any microorganisms in the sample on the membrane surface. A substrate is added that is enzymatically cleaved by any viable organism present. Only viable cells with intact membranes have ability to achieve this cleavage and retain the fluorescent label. This cleavage produces a fluorochrome that is retained within the cytoplasm of single cells, including spores. The membrane surface is then analyzed in the ChemScan RDI instrument where a laser scans the entire surface of the membrane in less than 3 minutes and detects all fluorescent events using sensitive photomultipliers.

Despite the impressive advantages offered by rapid microbiological methods, the progress in introducing these technologies into parenteral manufacturing and quality control operations has been slow. One reason could be the lack of clear guidance as to establishing the suitability of rapid methods as a replacement for current methodology. Another factor could be the enhanced sensitivity of rapid methods relative to old methodology—some have voiced concerns that, as a result, microbial limits would have to be raised, and there is a perception that regulatory agencies would not accept this. Another factor could be that pharmaceutical companies are focused on cost containment and headcount control, and this culture is not consistent with development and implementation of new technology. Another may be that industry, as a whole, is uncertain as to just how to go about gaining regulatory approval for improved microbial test methods. Regardless of the reasons for hesitancy, there is no denying that efficient pharmaceutical manufacturing is a critical part of an effective healthcare system, and the industry must make development and implementation of improved microbial test methods a priority. The changing regulatory environment presents the industry with an opportunity to aggressively pursue these technologies.

With the advent of process analytical technologies and the need for information faster without waiting for days, rapid microbiology systems have evolved and will continue to gain greater application in the parenteral industry. FDA guidelines for aseptic processing state that "other suitable microbiological test methods (e.g., rapid test methods) can be considered for environmental monitoring, in process control testing, and finished product release testing after it is demonstrated that the methods are equivalent or better than traditional methods