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Hideharu Shintani*

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***Corresponding author:** Hideharu Shintani, Faculty of Science and Engineering, Chuo University, 1-13-27, Kasuga, Bunkyo, 112-8551, Tokyo, Japan, Tel: +81425922336; Fax +81425922336; E-mail: shintani@mail.hinocatv.ne.jp

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Abstract

There are several well-developed rapid microbiological methods now becoming available that may have useful applications in pharmaceutical and medical devices. They are ATP bioluminescence, fluorescent labeling, electrical resistance, and nucleic acid probes. In choosing to employ rapid methods, the microbiologist should examine their prospective performances against the specific requirements for that sector. Some methods may require expensive equipment and offer full automation, and others represent only a small investment. The regulatory view of these methods is changing and they still officially have not been approved in medical and pharmaceutical area, but it will still be up to the microbiologist to demonstrate that the method chosen is fit for the purpose intended.

Keywords: Rapid microbiological methods; Bioburden, Pharmaceuticals; Medical devices

Introduction

Traditional microbiological methods of detection, enumeration, and identification using mostly culture methods are so often time-consuming and labor-intensive. These practical considerations often limit the extent to which microbiological tests are routinely applied both at the formulation development stage (i.e., preservative screening) and for Microbiological Quality Assurance (MQA). In the latter instance, the inevitable time delay associated with incubation often determines that MQA data are only of retrospective value. Pharmaceutical and medical device production and economic pressures can no longer accommodate this delay [1-3]. Considerable benefit would therefore be gained from the introduction of suitable, more rapid methods of microbiological analysis to the pharmaceutical and medical device sector. For these purposes, this review article has been prepared. The typical requirements for such rapid methods are summarized below.

- Rapid
- Sensitive
- Broad spectrum detection
- Potential for specificity
- Identification
- Viability assessment
- Simple
- Potential for automation
- Reproducible
- Compatible with sample matrices

However, they are not always suited to pharmaceutical and medical devices due to the large difference in the number of contaminating microorganisms. Their procedures of detection may be direct, in which individual microorganisms or populations of organisms are directly observed, or indirect, whereby microbial metabolism, metabolites, or components can be monitored. Some methods may be highly developed with extensive equipment and information support, while others can still be considered to be at relatively early stages of research or currently developed for only a narrow application range. Only a few appear able to meet the challenges of pharmaceutical and medical device microbiology [1-4]. It is also important to remember that the term "rapid" is variously applied to techniques of 5 min to 24 h duration˚ 5m tensive a e– de p d

Method	Detection Principle
Direct	
Fluorescent labeling	Stain microorganisms using a viability-indicating fluorophore; direct enumeration, usually after filter capture, by light excitation (epifiuorescent microscopy or laser scanning) and image analysis
Indirect	
ATP bioluminescence	Light emission from microbial ATP by luciferin/luciferase reaction. Amenable to amplification by intracellular adenylate kinase
Carbon dioxide detection	Monitoring of microbial metabolism using 14C-radiolabeld substrate to produce 14C-labeled carbon dioxide. Infrared CO_2 detection offers a more acceptable substitute
Chromatographic analysis	Detection of microbial metabolites and cellular components; gas chromatographic analysis of microbial fatty acid has been employed in identification
Dye reduction	Monitoring microbial metabolism of specified substrates by color changes in redox dyes; can form the basis of identification profile
Electrical resistance	Measurement of electrical changes (conductance, impedance) in specialized media due to microbial growth; enumeration based on time to exceed a specified detection level
Enzyme monitoring	Detection of microbial enzymes. By using appropriate substrates can form the basis of identification profiles
Šã{ ˘|˘•Áæ{ [^à[&ˆc^Álysate	Detection of (principally) Gram-negative bacterial lipopolysaccharide by gelation or colorimetric reaction
Nucleic acid probes	Labeled DNA or RNA probe hybridization to specific target sequences. Amplification of target by the polymerase chain reaction (PCR) increases sensitivity; competitive quantitative PCR offers enumeration
Phage-interaction technology	Host-specific bacteriophage infects target cells leading to phage DNA replication. Detection by expression of new protein (using recombinant phage) or cell lysis

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types of pharmaceutical and medical device application, although some manufacturers are now seeking methods applicable to a collection of related products. It is unlikely that any rapid method can be immediately applied in a wide range of situations without first undertaking extensive protocol development. The sensitivity of all methods can be enhanced by sample enrichment but this will lead to an inevitable increase in analysis time; additionally, contaminants grow at different rates and this may result in a substantially different microbial flora from the original sampled product. In sterility testing, where the bioburden is quite likely to be low, rapid methods generally require sample enrichment or extended incubation period to reach the microbial levels required for detection, which significantly differs from rapid methods used in the food facilities. Food facilities have approved the use of rapid methods to detect microorganismm rganismm rgani

Process Hygiene

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