



Making the Jump to **High-Throughput Microbial Detection**

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INTRODUCTION

The ability to rapidly and reliably detect microbial contamination is paramount for product safety markets including: food and dairy, environmental screening (water and air), personal care product testing, toxicology screening, and pharmaceutical testing. Conventionally, these tests have been conducted manually using standard plate count methods, with minimal throughput and high variability. Recently, there has been extensive effort to develop alternative, rapid methods to replace the slow and tedious traditional methods.

By definition “rapid methods” involve a reduction in assay time compared to standard

plate counts, usually through shorter incubation (growth) requirements. Progressing this further, the most advanced of these systems enable high-throughput programs by using 96-well microplate formats and automated plate handling systems. Although there are automated systems for Petri dish handling, the switch to microplate formats provides the biggest jump in throughput. In order to acquire sufficient information from a single well in a 96 well plate the object recognition limits need to be significantly smaller than that detected manually. In essence the entire plate count process needs to be miniaturized in order for it to be high-throughput. This article summarizes the current technology that is

driving the transition to high-throughput microbiology and discusses the implications of these advancements for food and product safety.

HIGH-THROUGHPUT TECHNOLOGIES

Several enabling technologies have emerged in the last decade that facilitate the transition to high-throughput microbiology. The major developments summarized below focus on rapid methods for detection of microbial contamination, although some of these methods can also be used for identification of specific pathogens.

MICROCOLONY DETECTION

High-resolution colony counting reliably detects colonies that are barely visible to the human eye. Consequently, the time requirement for growth enrichment is greatly reduced. Also advantageous, these methods are non-destructive and thus allow downstream identification of the organism. These methods use advanced illumination techniques to distinguish microcolonies (<25 µm) from the background. Since these techniques detect colonies, rather than single cells, growth is required. This precludes the detection of viable non-culturable organisms, but permits proliferation assays such as antimicrobial susceptibility testing. Microcolony resolution provides the means to detect organisms much earlier than with standard plate count methods. Thus testing procedures are accelerated and product is released earlier.

The ability to detect microcolonies permits the use of high-throughput microplate formats. In fact microcolony systems capture as much information from a single well (~5mm) in a 96-well plate as traditional plate counting yields from a 100mm Petri dish. In addition microplate formats greatly simplify data management, tracking and archiving. The resulting increase in data collection means that statistical robustness is improved and product and testing confidence is assured.

ATP BIOLUMINESCENCE

ATP bioluminescence measures light output produced by a luciferase reaction that is de-

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pendent on ATP released from microbial cells or ATP generated by bacterial adenylate kinase. ATP bioluminescence systems significantly reduce incubation requirements for growth and enrichment compared to standard plate count methods, although sample processing is more involved. In addition, this method is destructive and therefore requires parallel samples to proceed with organism identification.

SOLID PHASE AND FLOW CYTOMETRY

Solid phase and flow cytometry systems detect microorganisms in a liquid sample either in flow, or captured on a solid surface by membrane filtration. Detection of the captured organisms relies on light output from a fluorescent viability dye or cleavage of an enzymatic substrate. The primary advantage of these systems is the ability to detect single cells, thus enabling the quantification of viable non-culturable organisms. This also allows the growth phase requirement for detection to be eliminated completely. However, just as with ATP bioluminescence, this methodology is destructive and again requires parallel sample preparation for downstream organism identification.

DIRECT EPI-FLUORESCENT FILTER TECHNIQUE (DEFT)

Like solid phase laser cytometry, DEFT relies on a fluorescent detection reagent for single cell detection. Organisms are isolated by membrane filtration either before or after exposure to the fluorescent reagent. Since single cells are detected, no growth is required. Depending on the detection reagent, viable non-culturable cells may be enumerated. A variation of this technique AB-DEFT, uses a fluorescently labeled antibody for detection, and therefore can be used to push this technology towards identification of the contaminating organism.

DEFT methods rely on microscopic imaging for data collection. Typically, in order to collect data for an entire sample multiple microscopic images covering the entire filter surface are digitally stitched together. Alternatively, a sample of the filter surface can be imaged, resulting in non-representative data reporting. Ultimate-

ly the most advanced instruments, that are capable of full membrane data collection with a single image, offer the best alternative.

NON-IMAGING BASED METHODS

In addition to the direct and indirect microbe visualization methods outlined above, several other microbial indicators have been established. These typically monitor a by-product or corollary of microbial metabolism. For example the metabolic production of CO₂ gas can be monitored through a pH indicator, or by direct measurement of head space pressure. Heat is another consequence of microbial metabolism that can be measured, typically through the use of a calorimeter. In addition, conductance and impedance in liquid samples

can be correlated to microbial growth. A common aspect of these non-imaging based methods is that the data is collected on samples in liquid solution. Therefore, the data is collective for the whole sample and enumeration of organisms is accomplished through an extrapolation algorithm.

APPLICATIONS FOR PRODUCT SAFETY

The scale of distribution of commodities, personal care products, and pharmaceuticals in modern society brings an inherent vehicle for the spread of disease. The possibility of pathogen contamination is intrinsic to the entire supply chain: manufacturing, processing, packaging and distribution. ▶

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Careful monitoring of product safety on every level is essential to prevent public harm.

High-Throughput Microbial Detection Sectors

- Personal Care Product Testing.
- Pharmaceutical Testing.
- Toxicology Screening.
- Food and Dairy Market.
- Environmental Screening.

Of primary concern in product safety is the detection of bacterial pathogens. Bacterial pathogens are ever-present, and become especially detrimental if allowed to proliferate. Unfortunately, processing and packaging often bring the opportunity for bacterial pathogens to flourish if not carefully monitored. Consider the impact of the recent deluge of contaminated meat incidents. The potential impact of these events increases as these sectors become increasingly global. However, the prevalence and impact of these events can be greatly diminished with frequent monitoring throughout the supply chain.

Conventionally, these tests have been conducted manually using standard plate counts. A central problem with the standard methods is the limited number of samples that can be easily processed. This restricts contamination monitoring to lot specific analysis and minimal sampling points. In contrast, high-throughput methods enable massive sample processing and more representative analysis. Some of the additional advantages for moving towards high-throughput methods are: reduction in assay time, increased accuracy through method standardization, and increased statistical robustness by enabling more replicates.



THE FUTURE OF SAFETY TESTING

In the last decade the number of approved high-throughput methods in the public safety sector has increased exponentially. This reflects a growing acceptance of the potential of these new technologies, as well as increased understanding among the agencies that regulate these sectors. Overall, the trend points towards global implementation of rapid methods that can be soundly validated in an industrial setting.

Recent product safety press releases have placed considerable pressure on suppliers to demonstrate product safety in a traceable manner. This trend is likely to increase consumer demands for certification of the safety

status of products and commodities. With this will come revisions to product label standards to include expanded information on product safety, such as the absence of specific pathogens, testing method used, supply chain tracking, etc. Thus, in due course high-throughput microbial detection systems will enable product safety to be traceably guaranteed from the source to the home. ■

Cellular Technology Limited develops and markets the BioSpot line of rapid microbial detection systems offering high-throughput microcolony, DEFT, and solid phase cytometry systems. Information can be found at www.immunospot.com or by contacting colony-counters@immunospot.com.

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