Case Studies – How Rapid Microbiological Methods Enable Operational Efficiencies, From Routine Product Quality Testing to Process Failure Investigations.

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INTRODUCTION

Manufacturers in the bioproduction industry are faced with ensuring their processes are under control to deliver both high yield and safe drugs. The quality of the incoming raw, water and semi-finished materials introduced into the production process are as critical as the quality of the final product to be released.[1] The sterility test as described in the harmonized pharmacopeias is a minimum 14 day test and not suitable for short-shelf life products or in support of process investigations.[2] Often, this test can be as long as 24 days for cell-containing samples from bioprocesses. Speed, sensitive and accurate test results are critical for both in-process quality control and release product testing. The need for speed, automation and objectivity has led to the design and development of alternative rapid microbiological methods (RMM) to be more and more adopted in the biopharmaceutical industry. Two industrial case studies are presented to demonstrate how 2 different RMM's – an automated growth-based technology (BACT/ALERT® 3D) and a solid-phase cytometry technology (SCANRDI®) – can be used for in-process quality and product release testing, as well as for out of specification process investigations.

OBJECTIVE

Demonstrate that implementing RMM's for routine product quality testing and quality investigational tools improves operational efficiency for bioproduction manufacturers.

CASE STUDY 1 – PROCESS QUALITY AND RELEASE TESTING

Technology Background

The BACT/ALERT 3D (BTA) Microbial Detection System provides an automated non-destructive growth-based rapid microbial alternative to the 14-day traditional test method and is capable of detecting a variety of aerobic and anaerobic microorganisms.[3] The BTA system utilizes a colorimetric sensor and reflected light to detect carbon dioxide dissolved in the culture medium that is produced by growing microorganisms (Figure 1). The reflectance light of each incubated bottle is read every 10 min providing a continuous growth monitoring and result in real-time.

The BTA method utilizes direct inoculation of ≤ 10 mL of product into the BTA culture bottle consisting of supplemented Tryptic Soy Broth (TSB) along with atmospheric conditions required for aerobic or anaerobic growth. The BTA culture bottles are available standard and with resin to neutralize residual antibiotics present in the sample.

The BTA system is flexible and modular allowing users to determine incubation temperature based on testing needs. The DUAL-T instrument allows the user to incubate inoculated culture bottles at 2 temperatures, typically 20-25°C (aerobic bottles) and 30-35°C (anaerobic bottles), in accordance to the temperatures outlined in the U.S. Pharmacopeia for sterility testing. The system can be configured with up to 6 Incubation Modules, each holding 240 bottles for a total capacity of 1,440 bottles.

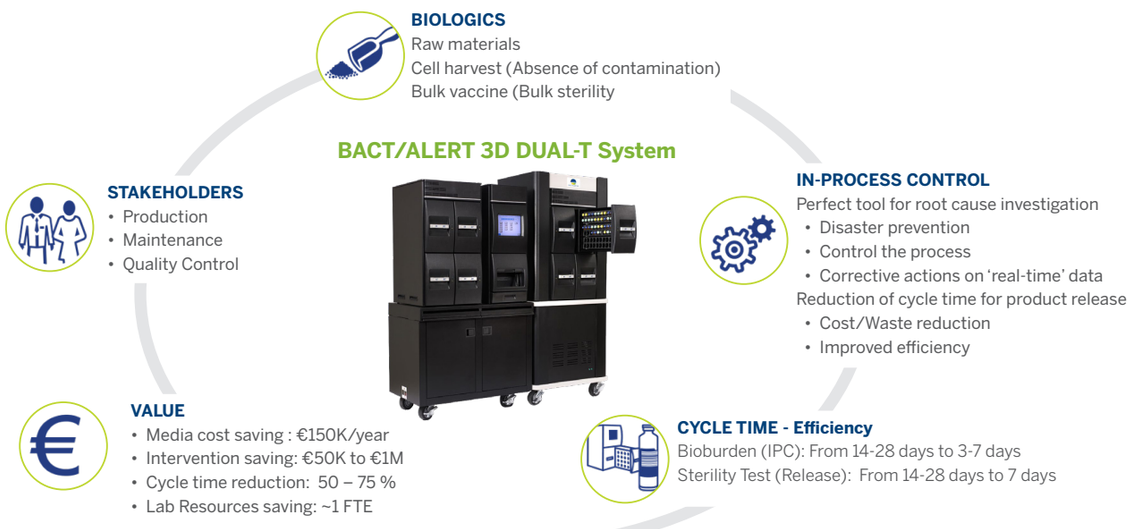
Figure 1: A. BACT/ALERT 3D technology uses a colorimetric sensor and reflected light to monitor presence of CO_2 dissolved in the culture medium. **B.** Liquid Emulsion Sensor changes from blue-green to yellow indicating a Positive result.

User profile: A multinational pharmaceutical **Company A*** manufacturing biologics, vaccines, medicines; and others.

Problem statement:

- High-valued complex biologics
- Conventional methods are manual and lengthy with time to results from 3 to 14 days. Samples containing cells from bioprocess testing for up to 24 days.
  - Not useful for in-process control
  - Limiting for final product release
- Controlling raw material, work in progress products and process quality to prevent downstream contamination.
- Weeks long investigations impacting production of at risk product and business risk.

Intervention: Validation and Implementation of BTA DUAL-T in routine testing for process and finished product quality.



CASE STUDY 2 – PROCESS INVESTIGATION

Technology Background

The SCANRDI is a rapid microbiology solution that uses a patented universal cell labelling and solid phase laser cytometry to identify viable microorganisms from filterable samples; offering an alternative to the traditional 14-day sterility test method. It is a non-growth based method that is able to detect bacteria, molds – both vegetative and sporulated - and yeasts, including stressed and fastidious organisms in Viable But Non Culturable (VBNC) state. The system sensitivity can detect a single cell within 3 hours.[4]

After sample filtration on a specially-designed membrane, the SCANRDI uses labeling non –fluorescent membrane permeant substrates which are cleaved by living cells by an enzymatic reaction, releasing fluorescent particles (free fluorochrome) within the cell. The cellular membrane holds the light-emitting fluorochrome within the cell and allows it to be detected by the SCANRDI Analyzer during the laser scanning step. The SCANRDI's laser scans the entire membrane in less than 3 minutes and immediately displays the total number of living cells fixed to the membrane. A scan map display showing the precise location of each organism for visual confirmation via the attached microscope system (Figure 2).

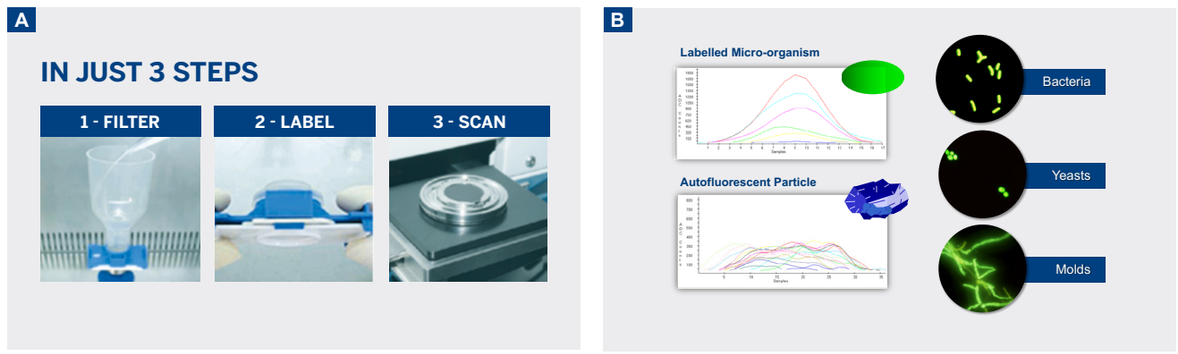


Figure 2: A. Sample filtration and preparation capture microorganisms on a specifically-designed membrane. **B.** Examples of graph results obtained on SCANRDI with microscope confirmation.

User profile: A multinational **Company B*** manufacturing vaccines, medicines and consumer healthcare products.

Problem statement:

- Sterile line filling maintenance and CIP quality
- Screening and monitoring quality of fill line and transfer in-process to prevent downstream contamination.
- Conventional methods are manual with lengthy time to results
- Weeks long investigations impacting production of at risk product and business risk.
- Need for rapid method to support investigation and quickly implement action plan.

Intervention: Use of SCANRDI to rapidly investigate process failures and implement corrective actions and monitor process quality.

CONCLUSION

Conventional methods are validated and widely accepted in the industry, however, these methods are cumbersome and lengthy; and some have never been validated. These case studies demonstrate the versatile applications of using RMM's in detecting early contamination in products and in the process - enabling the implementation of corrective actions, avoiding production downtime and release of contaminated products. These case studies demonstrate how routine use of RMM's enables manufacturers to improve their operational efficiency and profitability through greater production capacity, reduced testing cycle time by 50-75% and reduced investigation time vs. conventional methods.

The implementation of RMM starts to gain more traction in the pharmaceutical industry.

REFERENCES

* Company A's and Company B's names and locations are kept confidential.

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