BIOMÉRIEUX

A Recognized Rapid Microbiological Method for Cell & Gene Therapy Products:
Regulation & Industrial Application

Your Ally in Advancing Quality

INTRODUCTION

Cell & Gene Therapies (C&GT) or Advanced Therapeutic Medicinal Products (ATMP) are innovative medicine developed to treat diseases such as cancer, rare diseases, and autoimmune disorders.

Those therapies, based on living cells, have short shelf lives, are available in low volumes and cannot be sterilized by filtration or irradiation. Microbiological examination of cell-based products is critical to ensure safety prior to patient infusion.

The harmonized chapters of the compendial sterility test method – USP <71>, Ph.Eur. 2.6.1, JP 4.06 – are not adapted for C&GT products due to three major constraints:

Time To Result

Method Subjectivity

Matrices Compatibility

Those drawbacks need to be overcome in order to assess the sterility of these therapies prior to transfusion, and rapid solutions must be found to release safe C&GT. The **BACT/ALERT® 3D DUAL-T**, an automated and rapid growth-based technology reduces the Time To Results (TTR) while keeping the highest level of performance and compliance for C&GT release. It is compatible with complex matrices and the automated reading ensures objective results.

In Europe, this automated **growth-based method is now recognized as compendial and can be used in lieu of the conventional 14-day sterility test** for C&GT products. This method is described in the Ph.Eur. Chapter 2.6.27 “Microbiological Examination Of Cell-Based Preparation.”

GENERAL REGULATORY STATUS

As previously noted, the current conventional sterility test method for short shelf-life C&GT drugs is not suitable due to several major constraints, including:

A 14-day incubation period	**A subjective human inspection method**	**A membrane filtration step not adapted**	**Non-filterable & Turbid products**

The use of a rapid and modern microbiological technology can lead to improved microbiological control and is accepted by regulators pending validation. When considered as an alternative, these methods must be fully validated to demonstrate their non-inferiority to the traditional method.

A number of guidelines have been published to support such validation efforts, such as:

- The PDA Technical Report 33 **"Evaluation, Validation and Implementation of New Microbiological Testing Methods"**
- The USP <1223> **"Validation of Alternative Methods"**
- The Ph. Eur 5.1.6 **"Alternative methods for control of microbiological quality"**
- The European Pharmacopoeia booklet **"Examples of validation protocols of the alternative microbiological methods according to chapter 5.1.6 "Alternative methods for control of microbiological quality"** (2018 Edition)

These guidelines explain the methodology and the parameters to include in the validation protocol (Table 1), and show validation strategy examples that were already implemented and in use in the industry.

Table 1: Parameters to be validated when using a qualitative alternative microbial test (presence/absence) for sterility testing.

Parameter	Qualitative Test
Accuracy	No
Precision	No
Specificity	**Yes**
Detection Limit	**Yes**
Quantification Limit	No
Linearity	No
Operational Range	No
Robustness	**Yes**
Suitability	**Yes**
Equivalence	**Yes**

REGULATORY EVOLUTION

With the emergence of rapid microbiology methods, recent specific non harmonized pharmacopoeia chapters focusing on **RAPID MICROBIAL TESTING** have been developed, specifically applicable for short shelf life products or cell-based preparations (C&GTs).

Ph.Eur. 2.6.27 "Microbiological Examination of Cell-based preparations" with new COMPENDIAL methods

In this chapter dedicated to cell based preparations, automated growth based methods such as BACT/ALERT® 3D DUAL-T are recognized as compendial methods. ONLY a confirmation of the suitability of the method for the given cell-based preparation must be performed.

- JP G4 "Microorganisms – Rapid Counting of Microbes using Fluorescent Staining" and "Microorganisms – Rapid Microbial Methods" both **with alternative methods**
- USP <1071> "Rapid Microbial Tests for Lease of Sterile Short-life Products: A Risk-based Approach"
- In 2021, the USP has developed a new general chapter that is open for comments: USP <72> "Respiration-Based Rapid Microbial Methods for the Release of Short Shelf Life Products" to include the respiratory technologies as **new compendial method** for short shelf life products (Pending publication)

Those chapters open the ground for the use of modern, and rapid microbiological detection methods – such as the BACT/ALERT® 3D DUAL-T. The selection of the method must be based on the manufacturing process, the characteristics of the product to be tested and it should be supported by a risk-based approach.

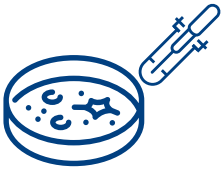
Method validation depends on the regulatory status, compendial or alternative methods. Demonstrating equivalence to the compendial traditional method is required for validating an alternative method.

VALIDATION OF RAPID METHODS

Four criteria are recommended to be investigated to build the risk-based approach to implement and validate a rapid sterility test method:

Sample Size	Time To Result	Limit of Detection	Specificity
Scarcity of C&GT/ ATMP product samples	Time To Patient is critical for short shelf life products	Sterility tests must show a LOD of 1 CFU	Ability to detect a wide range of microorganisms



Recognized existing and validated Rapid Sterility technologies that meet these requirements are routinely used in QC Laboratories of some pharmaceutical manufacturing sites, such as the BACT/ALERT® 3D DualT system.

BACT/ALERT® 3D DUAL-T DESCRIPTION

The **BACT/ALERT® 3D DualT** System includes both low (20-25°C) and high (30-35°C) incubation modules. It utilizes a colorimetric sensor and reflected light to monitor the presence and microbial production of CO2 dissolved in the culture medium (Figure 1). Microorganisms produce CO2 as they metabolize the substrates in the culture medium, causing a color change in the gas-permeable sensor at the bottom of each culture bottle, shifting it from blue-green to yellow (Figure 2).

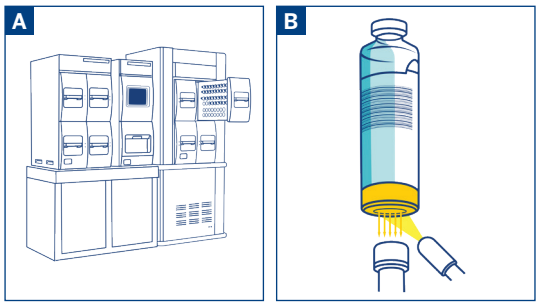


Figure 1: A. BACT/ALERT® 3D DUAL-T System B. The technology uses a colorimetric sensor & reflected light to monitor presence of CO_2 dissolved in the culture medium.

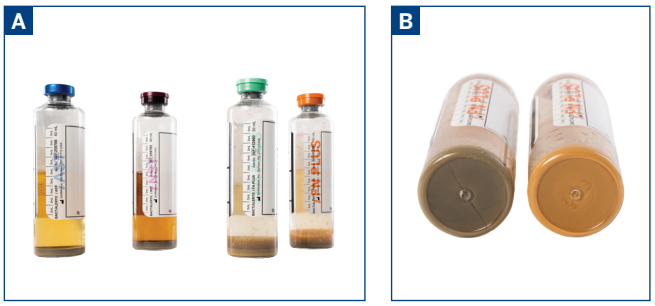


Figure 2: A. BACT/ALERT® culture media bottles. B. Liquid Emulsion Sensor changes from blue-green to yellow indicating a Positive result.

BACT/ALERT® 3D RESULTS

Industry studies have shown the Limit of Detection (LOD)[1] of the BACT/ALERT® 3D system to be 1 colony-forming units (CFU) and its Specificity[2] to be equivalent to the conventional sterility test. Overall, several studies have shown its LOD, Specificity, Robustness, Ruggedness and Equivalence to the membrane filtration sterility test method.

One of the first forays in the field of C&GT of the BACT/ALERT® technology was in the Quality Control of Platelets prior to transfusion that started back in 2005. With platelet products, TTR is of critical importance since the shelf life of platelets is between 5 to 7 days and platelets can't be sterilized and filtered, thus can't be tested for sterility according to the conventional 14 day sterility test.

A review of 11 peer-reviewed publications[3] (Table 2) has shown an average TTR of the BACT/ALERT® 3D system with its associated culture media bottles BPA/BPN of 18,6 hours (ranging from 8,3 to 89 hours) over 91 different commensal and environmental strains, in seeded platelet bags at 9,5 cfu/mL on average (ranging from 1 to 33 cfu/mL).

TTR (hours)	<24	<48	<72	<96
# of strains detected	80	85	86	91
% of strains detected	88%	93%	95%	100%

Table 2: Summary data of the TTR in 11 publications of seeded studies over 91 different microorganisms in platelet bags.

BACT/ALERT® 3D DUAL-T RESULTS

An evolution of the BACT/ALERT® 3D technology was the development of the BACT/ALERT® DUAL-T system and associated culture media bottles iAST/iNST & iFA PLUS/iFN PLUS. This platform was designed to meet pharmaceutical compendial testing standards, automatically growing and detecting potential contaminations of sterile pharmaceutical products, by direct inoculation with sample volumes up to 10mL and managing two incubation temperatures (20-25°C and 30-35°C).

Here, amongst the vast availability of scientific publications, we will describe 2 recent specific studies on the use and validation of the BACT/ALERT® 3D DUAL-T for the quality control of C&GT / ATMP, and Biotechnology products.

2019 publication from the United States NIH:

118 strains were tested, from which a panel of 110 strains was selected in the following analysis, using a mix of pharmacopoeia strains and challenge organisms representative of the NIH cGMP environment. This panel has shown an average TTR of the BACT/ALERT® 3D DUAL-T system of 60,8 hours – 2,5 days (Table 3) – and a detection of over 89% of all strains in less than 5 days (120 hours) when associated with its culture media bottles iFA PLUS/iFN PLUS incubated at 22,5°C and 32,5°C[4].

TTR (hours)	<24	<48	<72	<96	<120	<144	<168	<192
# of strains detected	24	60	81	92	98	102	104	110
% of strains detected	22%	55%	74%	84%	89%	93%	95%	100%

Table 3: Summary data of the TTR for a panel of 110 NIH strains on the BACT/ALERT 3D DualT.

In this study, the BACT/ALERT® DUAL-T technology has been shown and validated to detect a wide range of micro-organisms in a timely manner compared to the conventional 14 day sterility test.

2017 publication from Kite Pharma / Gilead:

Kite Pharma presented a poster at a PDA conference on their validation strategy of the BACT/ALERT® 3D DUAL-T system with its associated culture media bottles iAST/iNST incubated at 22,5°C and 32,5°C.

20 USP, EP & Environmental Isolates were evaluated to determine the Specificity and the LOD of the platform. As part of the rapid sterility test protocol, anaerobic organisms were evaluated at 32,5°C and aerobic organisms were evaluated at both 22,5°C and 32,5°C. This panel has shown an average TTR of the BACT/ALERT® 3D DUAL-T system of 40,7 hours – 1,7 days (Table 4) – and a detection of 100% of all strains in less than 5 days (120 hours)[5].

TTR (hours)	<24	<48	<72	<96	<120
# of strains detected	6	14	16	19	20
% of strains detected	30%	70%	80%	95%	100%

Table 4: Summary data of the TTR for a panel of 20 strains from Kite on the BACT/ALERT 3D Dual-T

In this study, the BACT/ALERT® 3D DUAL-T technology was determined to be equivalent to the compendial method with a TTR seven days or less. In EP 2.6.27 it is clearly stated that incubation should be no less than seven days, and the real time continuous monitoring of the system allows for early intervention and improved patient safety.

CONCLUSION

Several recent validation studies[4,5] have provided data comparing the BACT/ALERT® 3D DUAL-T System to the compendial sterility test method and enabled the adoption of this technology as a compendial method for microbiological examination of cell-based therapies in Europe, and in the US pending publication of general chapter USP<72>.

This enables the industry to replace the historical and conventional 14 days incubation compendial sterility test by a rapid and automated method, which results in greater patient safety by providing final sterility results in less than 14 days, cutting by half the total incubation time: over all the studies presented, 95% of the 221 strains are detected within 5 days.

However, for broader adoption of Rapid Microbiological Methods (RMM), a more rapid evolution of Pharmacopoeias to confer compendial status to rapid methods, and the harmonization of the applicable compendial chapters will be necessary to allow the industries to implement those technologies without the burden of the full validation for each of their products.

REFERENCES

1. Verdonk et al. The Most Probable Limit of Detection (MPL) for rapid microbiological methods J. of Micro. Methods Volume 82 (2010) 193-197

2. Ana Fonseca (Novartis) Sterility Testing Validation Using the BacT as a RMM – A Case Study 2019 PDA Practical Applications of Sterile Manufacturing Workshop March 13 - 14, 2019

3. List of the 11 publications on Blood Banks available on demand (cf. email)

4. England, et coll. Comprehensive Evaluation of Compendial USP<71>, BacT/ Alert Dual-T, and Bactec FX for Detection of Product Sterility Testing Contaminants J. of Clin. Microbiol. Volume 57 (2019) Issue 2

5. Kite Pharma / Gilead PDA 2017 poster BacT/ALERT 3D Dual-T as an Alternative Sterility Testing Method for Cell Therapy Products

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