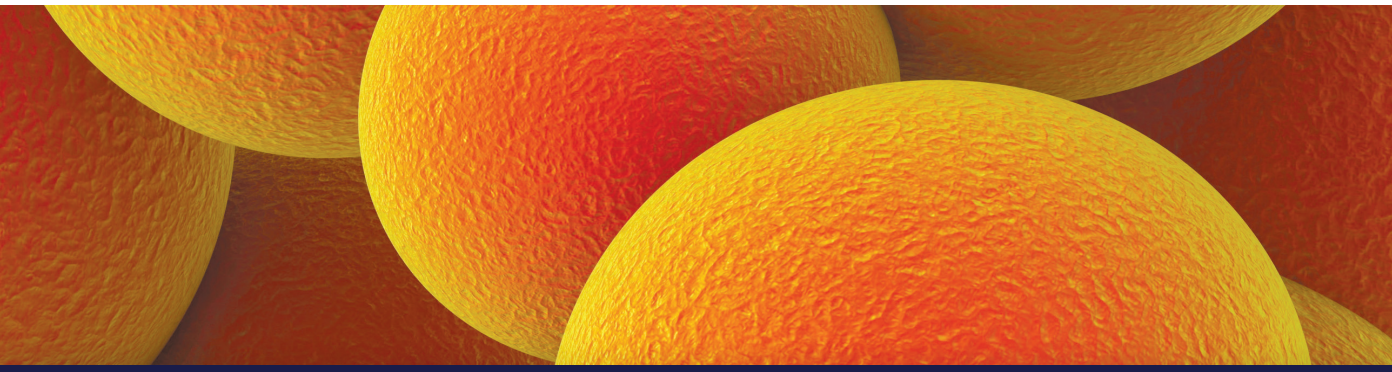
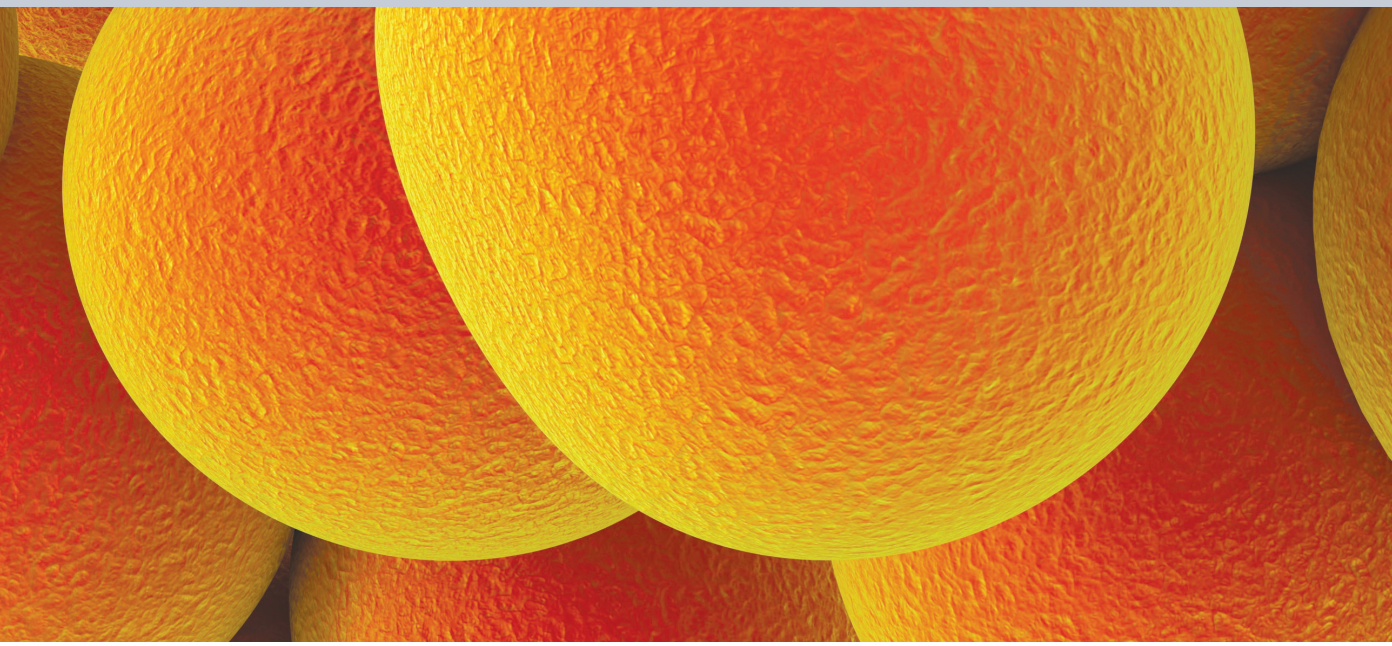
BioProcess™
SPECIAL REPORT



U.S. Approval of Three Rapid Microbiological Methods for MACI Product Release

by John Duguid, Ph.D., *Vericel Corporation*



ThermoFisher
SCIENTIFIC

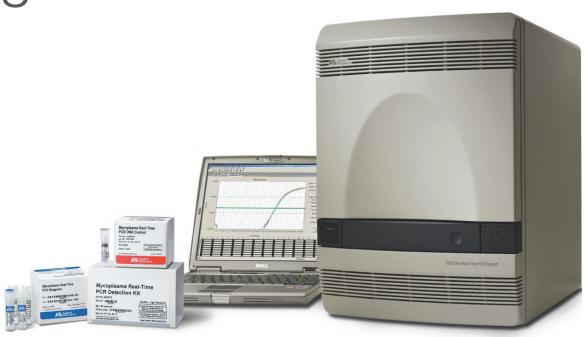
bioproduction

appliedbiosystems

Accelerate your cell culture lot release

Designed for purpose, confirmed in validation, accepted by regulatory agencies

With the Applied Biosystems™ MycoSEQ™ Mycoplasma Detection System, you can get results in hours, not days. You can also have the confidence that following validation, regulatory filing, and review, our customers have received regulatory acceptance to use the MycoSEQ system for lot-release testing applications. Regardless of your manufacturing process—cell culture, cellular therapy, tissue therapy, and more—get up to speed with the MycoSEQ system.



Find out more at **thermofisher.com/mycoseq**

ThermoFisher SCIENTIFIC

Autologous Cell Therapy Product Release

US Approval of Three Rapid Microbiological Methods

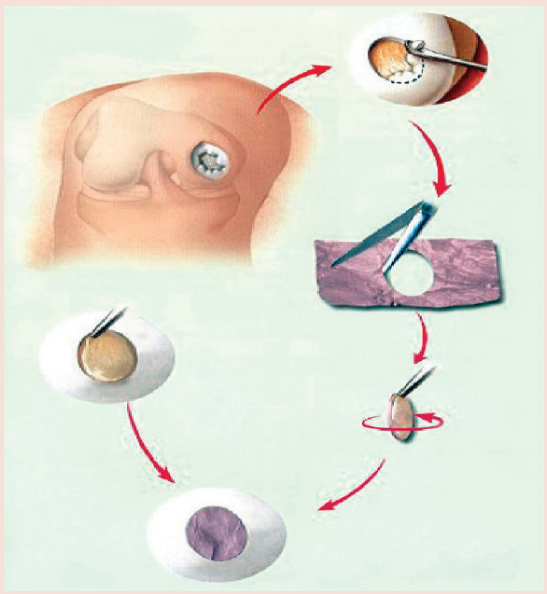
John Duguid

Short time frames are a major challenge in developing alternative microbiological methods for autologous cell therapy products. Ideally, results are made available in under a day. Obtaining regulatory acceptance also can be a challenge, but it is made easier if methods are included in an application (e.g., a biologics license application, BLA) rather than changing a method that is already part of an approved process. Comparing different detection platforms can be a challenge if they have different readouts, and validation packages are often extensive.

Background

The Vericel manufacturing facility located in Cambridge, MA, manufactures three commercial cell therapy products: Epicel® skin grafts for severe burns, Carticel® first-generation cartilage repair, and now MACI® third-generation cartilage repair. The company has been supplying cell therapy products commercially for over 20 years, having obtained the first BLA approval of any cell therapy product (Carticel) in 1997. That previous regulatory experience helped with the launch of the latest product. In 1999, Vericel began work on rapid microbiological methods to ensure the safety of its cell therapies, which have shelf lives as short as 24 hours.

Figure 1: MACI® autologous cultured chondrocytes on porcine collagen membrane

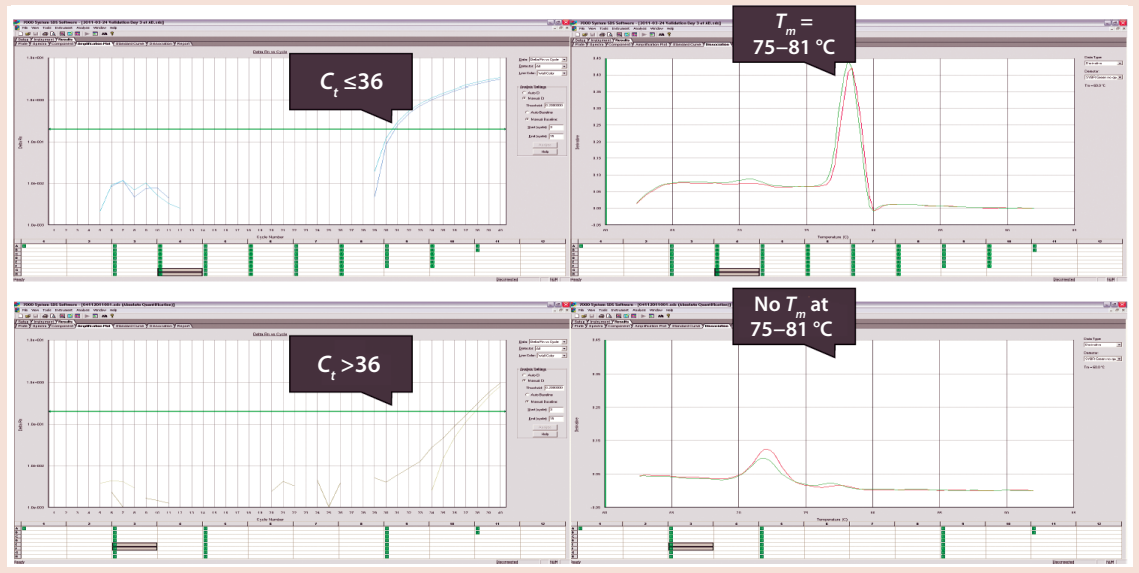


The Carticel autologous cultured chondrocyte treatment was on the market before the US Food and Drug Administration (FDA) issued regulations for cell therapy products. Vericel worked with the agency to establish an appropriate regulatory framework for the Carticel BLA, which included a postapproval clinical study. The most recent BLA approval for MACI included a prospective clinical trial, however. The SUMMIT trial evaluated the effectiveness of MACI in a two-year prospective, multicenter, randomized, open-label, parallel-group study.

MACI Manufacturing

The MACI manufacturing process essentially is just an extension of that for Carticel manufacturing, which supplies a suspension of cultured chondrocytes in a vial. The MACI

Figure 2: Mycoplasma positive (TOP) and negative (BOTTOM) results



process supplies cells loaded onto a porcine collagen membrane that facilitates surgical implantation (Figure 1). With the new product, a surgeon simply cleans up a patient's cartilage defect, cuts a MACI membrane to fit, and then implants the membrane cell-side down with fibrin glue. That simplifies the Carticel procedure, which necessitated a larger incision to allow for suturing of periosteum and injecting the cells underneath. Initial response to the simplified procedure from orthopedic surgeons has been positive overall.

A Risk-Based Approach: Autologous cell therapies are personalized medicine products and high-volume by nature. Each patient represents a unique product lot requiring full compliance with current good manufacturing practice (CGMP) requirements. Those include the same types of batch records, documentation, testing, and release as for any typical pharmaceutical product. In addition, shelf life is limited, so tests need to be performed on every lot as soon as possible after product is manufactured, preferably all on the same day.

Contamination control is a significant challenge in autologous cell therapy manufacturing. Routes of contamination include patient-specific biopsy source material, raw materials, and personnel. Strict CGMP compliance and manufacturing controls minimize the risk of microbial, mycoplasma, and endotoxin contamination in these biomanufacturing processes. Patient lot segregation, environmental monitoring and control, and aseptic process validation minimize the likelihood of introducing microbial contamination into a patient culture. A robust raw material inspection program requires sterility, mycoplasma, and endotoxin testing before materials can be used in a process. Highly manual processes require staff to work with small lots and numerous open manipulations in biosafety cabinets. A rigorous training program and strict adherence to standard operating procedures (SOPs) minimize the potential of contamination from personnel. Process design builds quality into each product because testing only provides an assurance of product quality but cannot create a quality product.

Because a traditional 14-day sterility test cannot be 100% complete at the time of product implantation, Vericel takes a risk-based approach to detect potential contamination and ensure the safety of its products. Sterility testing is performed at different points throughout manufacturing: on in-process samples before cryopreservation of primary and expansion cell cultures, again about three days before release to cover most aseptic manipulations, and finally on each final product assembled. Even the rapid final sterility test takes seven days, and products typically are implanted into a patient's knee within two to four days, so that test is not complete at the time of treatment. Endotoxin and mycoplasma tests are both same-day lot release tests that must be complete before product shipments, however.

Table 1: Mycoplasma assay validation

Parameter	Samples	Acceptance Criteria	Results
Specificity	Unspiked	No mycoplasma detected	6/6 negative
	Mycoplasma DNA	Detection in spiked samples	6/6 positive replicates for six species
Detection limit	Mycoplasma DNA	Detection in spiked samples	6/6 positive replicates for six species
	Mycoplasma <10 CFU/mL	Detection in spiked samples	6/6 positive replicates for six species
Repeatability	Unspiked	All replicates negative	24/24 negative
	Mycoplasma DNA	All replicates positive	24/24 positive replicates for six species
Ruggedness	Analyst to analyst	Δ(Average C_t) < 3	Δ(Average C_t) = 0.1
	Instrument to instrument	Δ(Average C_t) < 2	Δ(Average C_t) = 0.1
	Reagent lot to reagent lot	Δ(Average C_t) < 3	Δ(Average C_t) = 0.0
	Laboratory to laboratory	Δ(Average C_t) < 4	Δ(Average *A. laidlawii* C_t) = 3.1 Δ(Average *M. arginini* C_t) = 0.2 Δ(Average *M. fermentans* C_t) = 1.2 Δ(Average *M. hyorhinis* C_t) = 3.4 Δ(Average *M. orale* C_t) = 1.4 Δ(Average *M. pneumoniae* C_t) = 3.1
Equivalence	*Mycoplasma orale* 7 CFU/mL	NAT positive ≥ PTC positive	NAT 6/6 and PTC 0/6 positive
	MACI SUMMIT clinical trial samples	NAT results = PTC positive	NAT 78/78 and PTC 78/78 negative

Three Approved Tests

Microbial testing is required at different points throughout a manufacturing process, but standard methods take too long to be useful for cell therapy products. The United States Pharmacopeial Convention's (USP's) compendial sterility test takes 14 days to complete; Vericel's method halves that to seven days. USP mycoplasma testing takes 28 days; Vericel's method has reduced that to about six hours. Ideally, all testing would be complete before lot release.

Endotoxin testing using the rapid method is simple to perform, making it ideal for a fast-paced quality control (QC) environment. The rapid test meets all requirements of the kinetic/chromogenic pharmacopeial method. A small cartridge contains the *Limulus* amebocyte lysate (LAL) reagent and standard endotoxin for the kinetic method. An analyst simply dilutes a sample, puts the cartridge into a reader, and presses "Go." For single samples, Vericel uses handheld Endosafe®-PTS™ units. Because each test can take 15 minutes, the company recently implemented an Endosafe nexgen-MCS™ multicartridge reader that can run five tests in parallel. That allows QC to test multiple samples in about half an hour by loading them sequentially.

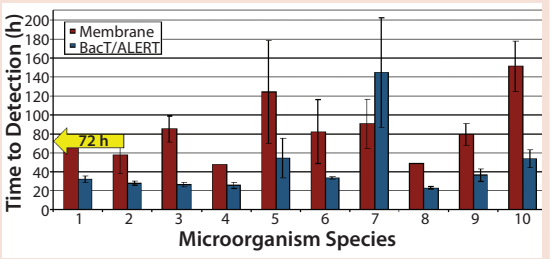
Because this rapid test method meets compendial requirements, it presents no regulatory hurdles to implementation. The method provides quantitative results in under an hour with a simple workflow. Vericel received European Medicines Agency (EMA) approval to use it for MACI manufacturing in 2013 and FDA approval to do so in 2016.

Mycoplasma: Mycoplasma contamination is rare in CGMP manufacturing facilities. It is a larger concern in research environments, where such infection of cell cultures goes largely undetected. CGMP facilities use appropriate cleaning procedures with mycoplasmacidal disinfectants and strict lot segregation to lower the risk considerably.

Vericel took a risk-based approach to identify the most appropriate technology for rapid mycoplasma testing of MACI products. An assessment identified nucleic-acid–based tests primarily based on polymerase chain reaction (PCR) as the most promising option. Numerous suppliers offer kits for rapid nucleic acid tests, and through risk assessment Vericel chose Thermo Fisher Scientific's MycoSEQ™ mycoplasma detection assay, which is based on real-time PCR and Power SYBR™ Green detection technology. The company subsequently validated the assay as part of its MACI development process.

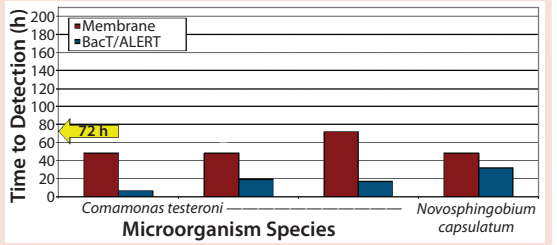
Mycoplasma Validation: The PCR-based assay has a straightforward workflow. After a cell lysing procedure, nucleic acids are extracted and purified for real-time PCR analysis. Figure 2 shows examples of MycoSEQ positive (top) and negative (bottom) results. Samples with a threshold value ≤36 and a melt temperature of 75–81 °C (above a certain derivative value threshold) are positive; samples with a C_t >36 and no melt-curve peaks between 75 and 81 °C are negative. Data analysis thus is straightforward.

Figure 3: Sterility assay validation



[1] *Staphylococcus epidermidis* [2] *Staphylococcus warneri*
[3] *Acinetobacter lwoffii* [4] *Pseudomonas fluorescens*
[5] *Streptomyces halstedii olivaceus* [6] *Candida parapsilosis*
[7] *Prioprionibacterium acnes* [8] *Clostridium sporogenes*
[9] *Penicillium chrysogenum* [10] *Aspergillus fumigatus*

Figure 4: Sterility case study



For validation of alternative microbiological methods, it is useful to engage regulators early and to understand their expectations. Vericel discussed its mycoplasma validation with FDA before submitting the MACI BLA, thus facilitating its use in the application. Table 1 summarizes the validation protocol and results. The validation study found the new method's specificity and limit of detection (LoD) to be equivalent to or better than the traditional culture method. The rapid PCR method detected mycoplasma in samples spiked with 10 CFU/mL, which the culture method did not detect.

Vericel also automated mycoplasma sample preparation using Thermo Fisher Scientific's AutoMate *Express*™ instrument. Total test time for the automated method is not much shorter than for the manual method, but it is unattended time that allows analysts to perform other tasks. Validation of the automated method found it to be slightly less sensitive than the manual method, but both methods demonstrated detection at or below 1 CFU/mL.

The total mycoplasma test time is under a day (five to six hours), and automation reduces cost. Outsourced testing using the culture method can cost as much as US$1,000 per test, whereas the in-house PCR method is about $100 per test. Vericel received EMA approval to use the new method for MACI testing in 2013 and FDA approval in 2016.

Sterility: Vericel began addressing the need for rapid sterility testing of cell therapy products in 1999 by discussing a validation protocol approach with the FDA. After a collaborative process with much agency involvement, final approval for a Carticel release test came in 2004.

The BacT/ALERT® 3D instrument automates growth-based microbial detection. Vericel chose a growth-based method as the first rapid method to discuss with the FDA because it was similar to the USP sterility test except for the detection platform. The instrument constantly agitates microbiological media, which enhances the growth of most species, and continually measures organism CO_2 production for rapid reporting of positive results.

Sterility Validation: Two Vericel validation protocols tested 14 individual microbial species over a five-year period. Figure 3 shows validation equivalence results from 10 species used in the final validation. The BacT/ALERT results in blue illustrate that, except for *Proprionibacterium acnes,* all organisms were detected in <72 hours, most in <48 hours. That is important to the safety of cell therapy products with short shelf lives. Validation requirements for alternative microbiological methods have evolved over time (since 1999), specifically with respect to detection limit. USP <1223> now defines multiple methods, including a most-probable number (MPN) approach followed by a chi-squared test or other statistical analysis to demonstrate equivalent microorganism detection. Subsequent comparison of BacT/ALERT with the USP sterility test found the BacT/ALERT detection limit to be equivalent or better.

The benefit of using a rapid sterility test became evident in 2005, when a contamination event affected four Carticel products. The BacT/ALERT method detected each one before product could be implanted into a patient. Even though the test isn't complete for seven days, most organisms are detected in enough time to interdict a contaminated product and prevent patient administration. Subsequent testing (Figure 4) found that the USP sterility test took 48–72 hours to detect the same species in these product samples, which probably would have been implanted before that detection. The true advantage of the rapid sterility test is this improved detection time, which improves patient

safety for products with short shelf lives. Vericel received FDA approval to use BacT/ALERT testing for Carticel products in 2004, EMA approval for MACI products in 2013, and FDA approval for the latter in 2016.

Compendial Work: The USP Microbiology Expert Committee is evaluating a rapid sterility test for potential inclusion in the compendium. Compendial methods are considered to be validated as written, so companies would not need to repeat method validation, but only demonstrate sample suitability similar to what is described in the current USP sterility test.

The committee commissioned a Modern Microbiological Methods Expert Panel in 2016 (I am a member) to define user requirements for a rapid test. Sample size and LoD are two particularly challenging requirements for the panel that have generated much interest and discourse. An official rapid test would be valuable for a number of stakeholder industries, cell therapy in particular.

A Cell Therapy Imperative

Using rapid microbiological methods for product testing and release is critical for autologous cell therapy products with short shelf lives. Regulatory acceptance is achievable in such cases and becoming more straightforward as more companies implement these methods. It is preferable to do so during product development and licensure because changes to applications for marketed products often require extensive comparability packages. An official compendial method certainly would facilitate more widespread adoption of such modern microbiological detection techniques.

Acknowledgments

Tony Cundell, Gary du Moulin, Grace Kielpinski, Sam Prinzi, Veenu Puri, and Barbara Seymour

John Duguid *is senior director of research and development at Vericel Corporation, 64 Sidney Street, Cambridge, MA 02139; 1-617-588-5555, fax 1-617-588-5554; jduguid@vcel.com.*

This article is based on a presentation he gave on the afternoon of 27 September 2017 at the BioProcess International Conference and Exhibition in Boston, MA. Endosafe is a registered trademark of Charles River Laboratories. BacT/ALERT is a registered trademark of BioMérieux.

bioproduction

Single use Cell culture Purification Analytics

RAPID MOLECULAR METHODS WHEN ACCURACY AND TIME-TO-RESULTS ARE CRITICAL

Get accurate results in hours, not days

There's too much at stake to take a risk when it comes to contaminant and impurity testing. That's why scientists turn to Applied Biosystems™ SEQ rapid molecular kits and systems. Discover the pharmaceutical industry's broadest portfolio of fully integrated molecular analytical solutions.

- Mycoplasma detection
- Viral detection
- Bacterial and fungal identification
- Glycan analysis
- Protein A and host cell protein quantitation
- Residual DNA

Explore our offerings at **thermofisher.com/pharmaanalytics**

ThermoFisher SCIENTIFIC