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Validation of an automated blood culture system for sterility testing of cell therapy products

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Abstract

Background aims. Automated blood culture systems are widely used for the detection of microorganisms in cell therapy products. However, they are not validated by the manufacturers for this purpose. The aim of this study was to assess the ability of the Bactec system (Becton-Dickinson, Le Pont-De-Claix, France) to detect the microorganisms that could contaminate cell therapy products. *Methods.* Three types of vials and conditions were tested: Plus Aerobic/F and Anaerobic/F media incubated at 35°C and Mycosis IC/F medium incubated at 30°C. All vials were incubated 10 days. We tested 18 microorganisms, including slow growers and some with fastidious nutritional requirements (10 bacteria, four yeasts, four filamentous fungi), each with four inocula ($10-10^4$ colony-forming units) performed in quintuplicate. *Results.* The combination of Plus Aerobic/F and Plus Anaerobic/F vials detected all the tested pathogenic bacteria, all the tested Gram-positive skin commensal or environmental bacteria, all the tested yeasts, and three of four tested filamentous fungi. The addition of the Mycosis IC/F vial extended the range of detected microorganisms to one fungal environmental contaminant. Two bacterial environmental contaminants were not detected by our method. Low inocula of the skin contaminant *Propionibacterium acnes* were detected only after 7 days of incubation. *Conclusions.* These data suggest that (i) the prolongation of the incubation time of Plus Aerobic/F and Plus Anaerobic/F vials from 7 to 10 days and (ii) the use of Mycosis IC/F medium make minor contributions in the sterility testing of cell therapy products. We have validated the Bactec method using aerobic and anaerobic vials incubated 7 days at 35°C.

Key Words: *automated blood culture systems, cell therapy product, microbial contamination, sterility testing*

Introduction

Bacterial contamination of cell therapy products is a potential source of morbidity and mortality in recipients. Possible sources of contamination include asymptomatic bacteremia of the donor at the time of collection and improper execution of aseptic technique during collection or processing. In France, cell therapy products are regulated by the National Agency for Medicines and Health Product Safety (ANSM), which emphasizes the importance of manufacturing products in a manner that prevents disease transmission and requires validated methods for testing products. The ANSM performs a nationwide survey of microbial contamination of cell therapy products annually. In 2011, this survey showed that among 17,578 cell products validated for distribution, 16 were contaminated with microorganisms. This rate increased to 15.4% depending on the type of cell products and the center (1–3). However, clinical sequelae after infusion of progenitor cells contaminated with bacteria are extremely rare (1–5), in part because of antimicrobial prophylactic regimens received by hematopoietic cell transplantation recipients (6).

The *European Pharmacopoeia*, in monograph 2.6.27, recommends methods based on cultures that detect bacteria and fungi without prescribing a specific method (7). Most clinical laboratories are

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equipped with automated blood culture systems (Bactec from Becton Dickinson, Le Pont-De-Claix, France, or BacT/Alert from Biomérieux, Craponne, France, or VersaTrek from i2a, Pérols, France) for the testing of patient samples. These automated methods are used to test cell therapy products in >91% of French laboratories (8). Automated blood culture systems are less labor-intensive and have a shorter time to detection, a higher rate of detection and a lower rate of false-positive results than the manual method (9–12). Although widely used, these techniques are not validated by the manufacturers for this purpose. Before its certification for sterility testing of cell therapy products, a laboratory must validate the method for the detection of a large panel of microorganisms over a large range of inocula. However, this procedure is expensive and time-consuming.

The *European Pharmacopoeia* defines the conditions under which the culture media are seeded and the validation of the control method and recommends the use of aerobic and anaerobic enriched culture media. If an automated blood culture system was used, the monograph recommends incubation at 35–37°C for at least 7 days (7). The culture conditions used for sterility testing of cell therapy products (i.e., type of vials, temperature, duration of incubation) differ among laboratories. Our laboratory followed the European recommendations, using a Bactec 9240 system with Bactec Plus Aerobic/F and Anaerobic/F media vials incubated at 35°C. For this study, we also inoculated a Mycosis IC/F medium vial incubated at 30°C. All the vials were incubated for 10 days. The aim of this study was to assess the ability of the Bactec system to detect the microorganisms that could contaminate cell therapy products.

Methods

Strains

The 18 different organisms representing gram-positive skin commensals, enteric organisms and environmental organisms for seeding were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), Collection de l'Institut Pasteur (CIP, Paris, France), Belgian Coordinated Collections of Micro-organisms (BCCM/IHEM, Brussels, Belgium) or the Centre de Ressources Biologiques Ferdinand Cabanne (CRB-FC, Besançon, France): *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6583, *Staphylococcus epidermidis* CIP 68.21, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* ATCC 13525, *Propionibacterium acnes* ATCC 6919, *Sphingomonas koreensis* CRB-FC 229.82, *Brevundimonas vesicularis* CRB-FC 54.27, *Corynebacterium amycolatum* ATCC 700207, *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 90028, *Aspergillus fumigatus* IHEM 22670, *Lichtheimia corymbifera* (formerly *Absidia corymbifera*) IHEM 16288, *Candida krusei* ATCC 6228, *Candida glabrata* IHEM 19160, *Cladosporium sphaerospermum* IHEM 18883 and *Penicillium chrysogenum* IHEM 22667. These microorganisms included slow growers and some with fastidious nutritional requirements. The strains were stored in brain heart infusion broth supplemented with 20% glycerol at −80°C until analysis.

Seeding of products

McFarland 0.5 standards (approximately 10^8 colony-forming units [CFU]/mL for bacteria and approximately 10^6 CFU/mL for yeast) were prepared in sterile saline solution with a nephelometer. For filamentous fungus, 1-week cultures on Sabouraud agar tubes were washed with 2 mL of saline solution. After vigorous shaking, the suspension in saline solution was taken and sedimented on the bench for 10 min. McFarland 0.8 suspensions (approximately 10^6 CFU/mL of spores) were prepared from the supernatant. Serial 10-fold dilutions were prepared in saline solution to obtain a stock suspension from which 10 mL (containing 10, 10^2, 10^3 or 10^4 CFU) was used to seed the vials. We also tested the time to detection with Bactec vials seeded with 10 mL of umbilical cord blood containing 10^4 CFU of the reference strains of *E. coli* and *S. epidermidis*. All assays were performed in quintuplicate, for a total of 1110 vials seeded. We controlled the quantity of microorganisms seeded by plating of 100 µL of the 10^3 CFU/mL dilution and counting on agar plates in duplicate. Organism quantification showed actual values of 62–218 CFU (mean, 138 CFU) for the target of 10^2 CFU.

Cultures

As previously described (9), vials were quickly inserted into the Bactec system and incubated for 10 days at 35°C (for Plus Aerobic/F and Plus Anaerobic/F) or 30°C (for Mycosis IC/F). Automated readings were taken every 10 min. Bottles detected as positive were sampled for Gram staining and sub-culture. The time of positivity (in hours) was noted. The identification of the sub-cultured bacteria and yeasts was confirmed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry with a Microflex LT spectrometer (Bruker Daltonik GmbH, Bremen, Germany), according to the manufacturer's instructions (13).