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Validation of the Microbiological Testing of Tissue Preparations Using the BACTEC™ Blood Culture System

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Keywords
Validation · Microbiology · Tissue

Summary
Background: Since blood culture bottles are validated by the manufacturer for blood only, an additional validation for the use with fluids of tissue preparations is necessary. **Methods:** Two 10-ml samples of cornea culture medium, histidine-tryptophan-ketoglutarate (HTK) solution, or Ringer solution at the end of femur head thermodisinfection were given into blood culture bottles (BD BACTEC™ Plus Aerobic/F, Anaerobic/F for cornea culture medium and BD BACTEC™ Standard Aerobic/ Anaerobic for HTK and Ringer solution) and subsequently spiked with 10–100 colony forming units (CFU) of bacteria or fungi (aerobic bacteria: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*; anaerobic bacteria: *Clostridium sporogenes*; fungi: *Candida albicans, Aspergillus brasiliensis*) according to the European Pharmacopoeia Chapter 2.6.1. Results: All tested bacteria and fungi could be detected in all solutions. All positive and negative controls were tested correctly. Compared to the positive controls, the microbial growth was delayed in the antibiotic-containing cornea culture medium, and negative in two cases of *B. subtilis* spiking. Conclusion: The use of BACTEC™ blood culture bottles seems to be a suitable method for microbiological testing of HTK solution, Ringer solution, and, with limitations, also for testing of the antibiotic-containing cornea culture medium.

Schlüsselwörter
Validierung · Mikrobiologie · Gewebe

Zusammenfassung
Hintergrund: Da Blutkulturflaschen vom Hersteller nur für den Einsatz mit Blut validiert sind, bedarf es einer zusätzlichen Validierung für Flüssigkeiten von Gewebezubereitungen. **Methoden:** Zwei 10-ml-Proben von Hornhautkulturmedium, Histidin-Tryptophan-Ketoglutarat (HTK)-Lösung und Ringer-Lösung am Ende der Femurkopf-Thermodesinfektion wurden in Blutkulturflaschen gegeben (BD BACTEC™ Plus Aerobic/F, Anaerobic/F für Hornhautkulturmedium, BD BACTEC™ Standard Aerobic/ Anaerobic für HTK- und Ringer-Lösung) und nachfolgend mit 10–100 colony Forming Units (CFU) von Bakterien und Pilzen (aerobe Bakterien: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*; anaerobe Bakterien: *Clostridium sporogenes*; Pilze: *Candida albicans, Aspergillus brasiliensis*) gemäß der Europäischen Pharmacopoeia Chapter 2.6.1. beimpft. **Ergebnisse:** Alle eingesetzten Bakterien und Pilze konnten in allen Flüssigkeiten detektiert werden. Alle positiven und negativen Kontrollen wurden korrekt getestet. Im Vergleich zu den positiven Kontrollen war das mikrobielle Wachstum in dem antibiotikahaltigen Hornhautkulturmedium verzögert und bei *B. subtilis* in zwei Fällen negativ. **Schlussfolgerung:** Der Einsatz von BACTEC™ Blutkulturflaschen scheint eine geeignete Methode für die mikrobiologische Testung von HTK-Lösung, Ringer-Lösung und mit Einschränkungen auch für die Testung von antibiotikahaltigem Hornhautkulturmedium zu sein.

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Introduction

The explantation, transport, and processing of tissues always carry a significant risk of microbiological contamination. Therefore, microbiological testing of tissue preparations is of great importance for the safety of the recipient.

Blood culture bottles are well established for the testing of blood and other body fluids (e.g. cerebrospinal fluid, ascites) of patients suspected of having an infection [1, 2]. Some systems contain special substances that inactivate antibiotics to enhance the sensitivity of microbial growth [3, 4]. Such professional test systems are easy available in high quantities for hospitals and microbiological institutions with a low expenditure of human labor at affordable costs. This makes blood culture bottles also an interesting test system for tissue banks. Since blood culture bottles are only validated by the manufacturer for blood and blood products, an additional validation for the use of fluids from tissue preparations is necessary. Such a validation should follow the guidelines of the European Pharmacopoeia because tissues and tissue preparations are under the regulations of the drug law in many European countries, as it is in Germany.

Material and Methods

The BD BACTEC™ culture vials (Becton Dickinson and Company, Sparks, NV, USA) were used. For our validation we followed the guidelines of the European Pharmacopoeia chapter 2.6.1. [5].

The following solutions, which are used in the daily routine of our multitissue bank, were tested:

i) Cornea organ culture medium with the following composition without and with 6% dextran 500 (as it is used for detumescence of organ-cultured corneas): 2,000 ml contain 200 ml minimal essential medium with Earl's salts (10×) ; 40 ml fetal calf serum (concentration of 2%), 20 ml penicillin/streptomycin 10,000 U / 10,000 µg/ml (final concentration of 62.5 µg/ml penicillin und 100 µg/ml streptomycin), 20 ml amphotericin B 250 µg/ml (final concentration 2.5 µg/ml), 20 ml L-glutamin (200 mmol/l), 25 ml HEPES buffer (1 mol/l), 58.6 ml NaHCO3 (7.5%), and 1,616.4 ml distilled water.

ii) Histidine-tryptophan-ketoglutarate (HTK) solution (Custodiol®, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) which is used to store and transport explanted cardiovascular tissues from the donor site to the tissue bank. 1 l made of distilled water contains 0.8766 g NaCl, 0.6710 g KCl, 0.1842 g potassium-hydrogen 2-ketoglutarate, 0.8132 g $MgCl_2$ 6 H_2O, 3,7733 g histidin, 0.4085 g trypotophan, 5.4615 g mannitol, 0.0022 g $CaCl_2$ 2 H_2O.

iii) Ringer solution (Fresenius Kabi GmbH, Bad Homburg, Germany): 6.5 g NaCl, 0.42gKCl, 0.25g CaCl2 2 H2O, 1 mol of NAHCO3 dissolved in 1 l of distilled water. The Ringer solution is used for thermal disinfection of femoral heads in the Marburg Bone Bank System 'Lobator SD' and was used after standard processing in our tissue bank as described before [6].

We used the BACTEC™ Plus Aerobic/F and Anaerobic/F culture vials for the cornea culture medium. This culture vials contain nonionic adsorbing and cationic exchange resins which inactivate antibiotics [4, 7]. BACTEC™ Standard Aerobic/F and Anaerobic/F culture vials were used for HTK and Ringer solution.

The following test microorganisms were used for this validation study:

- Aerobic bacteria
  - *Staphylococcus aureus* ATCC 6538
  - *Bacillus subtilis* ATCC 6633
  - *Pseudomonas aeruginosa* ATCC 9027
- Anaerobic bacteria
  - *Clostridium sporogenes* ATCC 19404
- Fungi
  - *Candida albicans* ATCC 10231
  - *Aspergillus brasiliensis* ATCC 164004

For all microorganisms a fresh overnight culture was prepared which was then diluted with thioglycollate medium to reach a final dilution of 100–1,000 colony forming units (CFU per ml. The dilution was performed using a photometry method. 100 µl of this solution were given into the blood culture bottles to have a spiking volume of 10–100 microorganisms.

At first 10 ± 1 ml of the different test solutions were transferred into the test vials using a syringe with a cannula under sterile conditions. The cornea culture medium was stored at 32 ± 1 °C for 3 days to simulate a common time point of its microbiological testing and to allow related changes during incubation. The Ringer solution as processing solution after femoral head thermal disinfection was stored after its preparation at –18 ± 2 °C until use and then defrosted and warmed to room temperature. The cooled HTK solution (6 ± 2 °C) was used directly from its original packing. As second step 100 µl of the microorganism solution was injected into the blood culture bottles, again under sterile conditions.

At least 2 test vials were spiked for all 4 different solutions. For *S. aureus* aerobic and anaerobic test vials were used. The test for *B. subtilis* was repeated completely once because of absence of microbiological growth in the cornea organ culture media in the first run. For every test microorganism two bottles were spiked with the solution only (negative control) and two with the microorganisms only (positive control). Altogether 88 blood culture bottles were included in this validation study.

All spiked blood culture bottles were stored at room temperature (20 ± 4 °C) for 12 h to simulate the transport from the tissue bank to the microbiological laboratory and then transferred to the BACTEC™ FX unit for incubation at 35 ± 1 °C and automatic reading using a CO_2/fluorescein method. The blood culture bottles were left in the BACTEC™ FX unit until the detection of microbiological growth or to a maximum of 14 days. The time point of microbiological growth was recorded automatically and later evaluated and calculated in hours after insertion into the BACTEC™ FX unit.

Through subculturing on blood agar plates or Sabouraud agar plates, the pure culture and the typical morphology of the colonies were proven for all positive blood culture bottles (20 µl liquid per bottle, incubation for 48 h for blood agar and 7 days for Sabouraud agar before evaluation). All negative test vials were examined the same way to prove absence of microorganisms using 100 µl of liquid.

Results

All results are shown in table 1, indicating the duration in hours until the microbial growth was detected by the BACTEC™ FX unit. All positive controls showed confirmed growth of the inserted test microorganism. All negative controls remained sterile. The liquid from all negatively tested spiked blood culture bottles did not show any microbial growth on the agar plates.

The detection rate of the test microorganisms in presence of HTK and Ringer solution did not differ significantly from the positive controls. The mean duration until growth of microorganisms was detected was 9.25 h in the positive controls

Table 1. Time in hours until microbial growth was detected in the BACTEC™FX unit for all different tested solutions (– indicates no growth)

Microorganism	Positive control	Cornea culture medium 1	Cornea culture medium 2	Cornea culture medium with dextran 1	Cornea culture medium with dextran 2	Positive control	HTK 1	HTK 2	Ringer 1	Ringer 2
Pseudomonas aeroginosa	5	13	11	10	9	4	4	4	4	4
Staphyloccocus aureus aerob	7	–	–	–	–	5	5	5	5	5
Staphyloccocus aureus anaerob	10	42	37	39	32	9	9	9	8	8
Bacillus subtilis 1	3	19	–	13	14	1	2	2	1	2
Bacillus subtilis 2	1	25	20	–	14	5	6	6	4	3
Clostridium sporogenes	10	16	17	19	19	9	9	9	57	10
Candida albicans	15	18	24	78	25	11	12	12	12	12
Aspergillus brasiliensis	18	30	15	14	14	30	29	21	15	14

(median 7 h, range from 1–30 h). This duration was on average 9 h for the HTK solution (median 7.5 h, range from 2–29 h). For Ringer solution the mean duration until a positive reading was recorded was 10.25 h (median 6.5 h, range 1–57 h).

For the cornea organ culture medium groups the duration until microbial growth was detected in the blood culture bottles was moderately longer compared to the positive controls. While this took on average 8.6 h in the positive control (median 8.5 h, range 1–18 h), the presence of cornea organ culture medium without dextran delayed this result to a mean of 22.1 h (median 19 h, range 11–42 h) and with dextran to comparable 23.1 h on average (median 14 h, range 9–78 h).

P. aeroginosa, *C. sporogenes*, *C. albicans*, and *A. brasiliensis* could be reliably detected in the presence of cornea organ culture medium. However, *S. aureus* was only found in the BACTEC™ Plus anaerobic culture vials and *B. subtilis* was only detected in 6 out of 8 spiked blood culture bottles.

Discussion

Blood culture bottles are a sensitive and established microbiological test system for patients suspected having septicemia [1]. They are also widely used for other liquid specimens such as cerebrospinal fluid or joint aspirate since they are easily available and ready to use. Furthermore, by using certain resins in the culture bottles also antibiotic-containing fluids can be evaluated with regard to microbiological contamination [3].

Therefore, this system might also be suitable to analyze fluids and media used for preparation and culture of human tissues such as bone or cornea.

In an initial validation study, we evaluated Ringer solution used for thermal disinfection of bone and HTK solution as transport medium for cardiovascular tissues in combination with BACTEC™ Standard Aerobic/F and Anaerobic/F culture vials.

The use of antibiotics in cornea organ culture medium is a helpful method to significantly reduce the microbiological contamination of donor corneas and to make them safe for the recipients [8]. Several studies have shown that donor corneas are significantly contaminated with microorganisms even after disinfection of the globe [9–11]. Therefore, penicillin-, streptomycin- and amphotericin B-containing cornea culture medium was examined in combination with BACTEC™Plus Aerobic/F and Anaerobic/F blood culture bottles with antibiotic adsorbing resins. Nzeako et al. [12] found a high inactivation rate of different antibiotics by the BACTEC™Plus resins up to a antibiotics concentration of 100 µg/ml. According to the European Pharmacopoeia guidelines we used *S. aureus*, *B. subtilis* and *P. aeruginosa* as aerobic bacteria, *C. sporogenes* as anaerobic bacteria, and the fungi *C. albicans* and *A. brasiliensis* [5].

All these microorganisms were detectable in all analyzed fluids using the BACTEC Standard or the BACTEC Plus Aerobic/F and Anaerobic/F blood culture bottles. However, we found the following limitations:

i) In cornea organ culture media the results were variable for *B. subtilis* for reasons we do not know.
ii) In the bottles with antibiotic-containing cornea culture medium a delayed detection of microorganisms was observed, which implicates that the antimicrobial substances, despite the presence of resins, inhibit the growth of the spiked microorganisms. Thuret et al. [13, 14] investigated the effectiveness of blood culture bottles for sterility testing of cornea organ culture medium for a wide selection of bacteria and fungi. The antibiotic-containing cornea organ culture medium eradicated 5 of 14 different tested bacte-

ria. The remaining 9 could be detected with a rate of 100% in the BACTEC™Plus Aerobic/F and Anaerobic/F blood culture bottles if a minimal spiking volume of 250 CFU per culture vial was used. 91% of 11 different spiked fungi (40 out of 44) with a minimal spiking volume of 10 CFU per culture vial could be detected using BACTEC™Plus Aerobic/F.

iii) S. aureus spiked in the antibiotic-containing cornea culture media could only be determined in the anaerobic culture bottles. We hypothesize, that additional factors like culture conditions might be crucial to reliably detect bacterial growth in media containing antimicrobial substances.

In a validation study Plantamura et al. [15] demonstrated a reliable and fast detection of all tested microorganisms in less than 48–72 h in cell culture medium without antibiotics using the BacT/Alert® sytem. With regard to the timespan of detection of microbial growth, their data were comparable with those obtained with our antibiotic-containing media.

In conclusion the results presented here show that blood culture bottles are suitable also for sterility testing of HTK and Ringer solution in compliance with the European Pharmacopoeia chapter 2.6.1. A fast and reliable detection for all test bacteria and fungi with no difference to the positive controls could be achieved.

We could also demonstrate a high detection rate of different microorganisms in antibiotic-containing cornea culture media within a short time span using BACTEC™Plus blood culture bottles. Since bacterial growth in this fluid seems to be somehow compromised, further investigation is needed to undoubtedly confirm the suitability of blood culture bottles for sterility testing of samples containing cornea organ culture medium.

Disclosure Statement

All authors declare no personal or financial conflict of interest.

References

1 Wilson ML: Blood cultures. Introduction. Clin Lab Med 1994;14:1–7.
2 Wilson ML, Weinstein MP: General principles in the laboratory detection of bacteremia and fungemia. Clin Lab Med 1994;14:69–52.
3 Wallis C, Melnick JL, Wende RD, Riely PE: Rapid isolation of bacteria from septicemic patients by use of an antimicrobial agent removal device. J Clin Microbiol 1980;11:462–464.
4 Applebaum PC: Enhanced detection of bacteraemia with a new BACTEC resin blood culture medium. J Clin Microbiol 1983;17:48–51.
5 European Directorate for the Quality of Medicines and HealthCare: European Pharmacopeia 7.1.; chapter 2.6.1. Strasbourg, EDQM Council of Europe, 2011.
6 Pruss A, Seibold M, Frommelt L, Garrel T, Gürtler L, Dörffel Y, Pauli G: Validation of the 'Marburg bone bank system' for thermodisinfection of allogenic femoral head transplants using selected bacteria, fungi, and spores. Biologicals 2003;31:287–294.
7 Flayhart D, Borek AP, Wakefield T, Dick J, Carroll KC: Comparison of BACTEC Plus blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J Clin Microbiol 2007;45:816–821.
8 Pels E, Vrensen GF: Microbial decontamination of human donor eyes with povidone-iodine: penetration, toxicity, and effectiveness. Br J Ophthalmol 1999;83:1019–1026.
9 Keates RH, Mishler KE, Riedinger D: Bacterial contamination of donor eyes. Am J Ophthalmol 1977;84:617–619.
10 Hudde T, Reinhard T, Möller M, Schelle C, Spelsberg H, Cepin A, Sundmacher R: Corneoscleral transplant excision in the cadaver. Experiences of the North Rhine Westphalia Lions Cornea Bank 1995 and 1996 (in German). Ophthalmologe1997; 94:780–784.
11 Albon J, Armstrong M, Tullo AB. Bacterial contamination of human organ-cultured corneas. Cornea 2001;20:260–263.
12 Nzeako BC, Al-Qasabi SS: Evaluation of the neutralising capacity of Bactec medium for some antibiotics. Br J Biomed Sci 2004;61:171–174.
13 Thuret G, Carricajo A, Chiquet C, Vautrin AC, Celle N, Boureille M, Acquart S, Aubert G, Maugery J, Gain P: Sensitivity and rapidity of blood culture bottles in the detection of cornea organ culture media contamination by bacteria and fungi. Br J Ophthalmol 2002;86:1422–1427.
14 Thuret G, Carricajo A, Vautrin AC, Raberin H, Acquart S, Garraud O, Gain P, Aubert G: Efficiency of blood culture bottles for the fungal sterility testing of corneal organ culture media. Br J Ophthalmol 2005;89:586–590.
15 Plantamura E, Huyghe G, Panterene B, Delesalle N, Thepot A, Reverdy M, Damour O, Auxenfans C: Validation of the BacT/ALERT® 3D automated culture system for the detection of microbial contamination of epithelial cell culture medium. Cell Tissue Bank 2012;13:453–459.