ORIGINAL ARTICLE

Direct blood culturing on solid medium outperforms an automated continuously monitored broth-based blood culture system in terms of time to identification and susceptibility testing

E. A. Idelevich, B. Grünastel, G. Peters and K. Becker
Institute of Medical Microbiology, University Hospital Münster, Germany

Abstract

Pathogen identification and antimicrobial susceptibility testing (AST) should be available as soon as possible for patients with bloodstream infections. We investigated whether a lysis-centrifugation (LC) blood culture (BC) method, combined with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification and Vitek 2 AST, provides a time advantage in comparison with the currently used automated broth-based BC system. Seven bacterial reference strains were added each to 10 mL human blood in final concentrations of 100, 10 and 1 CFU/mL. Inoculated blood was added to the Isolator 10 tube and centrifuged at 3000 *g* for 30 min, then 1.5 mL sediment was distributed onto five 150-mm agar plates. Growth was observed hourly and microcolonies were subjected to MALDI-TOF MS and Vitek 2 as soon as possible. For comparison, seeded blood was introduced into an aerobic BC bottle and incubated in the BACTEC 9240 automated BC system. For all species/concentration combinations except one, successful identification and Vitek 2 inoculation were achieved even before growth detection by BACTEC. The fastest identification and inoculation for AST were achieved with *Escherichia coli* in concentrations of 100 CFU/mL and 10 CFU/mL (after 7 h each, while BACTEC flagged respective samples positive after 9.5 h and 10 h). Use of the LC-BC method allows skipping of incubation in automated BC systems and, used in combination with rapid diagnostics from microcolonies, provides a considerable advantage in time to result. This suggests that the usefulness of direct BC on solid medium should be re-evaluated in the era of rapid microbiology.

Keywords: Antimicrobial susceptibility testing, bacteraemia, bloodstream infections, detection, direct blood culturing, identification, rapid diagnostics, sepsis, solid medium
Original Submission: 2 October 2015; **Revised Submission:** 14 December 2015; **Accepted:** 15 December 2015
Article published online: 23 December 2015

Corresponding author: K. Becker, University Hospital Münster, Institute of Medical Microbiology, Domagkstrasse 10, 48149 Münster, Germany
E-mail: kbecker@uni-muenster.de

Introduction

It is well recognized that sepsis mortality can be considerably lowered if adequate antimicrobial therapy is administered early, ideally in the first hour after onset of symptoms [1]. Obviously, at this very early time point, the causative agent is unknown and the therapy can only be chosen empirically. Nevertheless, rapid microbiological diagnostics still provide significant benefit for patients by shortening the time to appropriate antimicrobial treatment [2,3], reducing mortality [4] and length of hospital stay [3]. Recently, the improvement of clinical outcomes due to rapid diagnostics has been confirmed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) -based identification [5–9]. Considerable advances have been achieved in rapid identification applying direct MALDI-TOF MS to positive blood cultures (BCs) [10] or performing MALDI-TOF MS on a tiny biomass grown very quickly (a few hours) on solid medium after sub-culturing from positive BCs [11–14]. However, incubation of blood samples in automated liquid medium BC systems remains a substantial time-limiting factor. This approach, using continuous

http://dx.doi.org/10.1016/j.nmni.2015.12.004

monitoring for detection of microbial growth, has been the reference standard for detection of microorganisms in blood for the last three decades [15,16]. Before the introduction of these systems, lysis-centrifugation BC (LC-BC) method was commonly used [17–19]. This method implements lysis of blood cells in a whole blood sample and centrifugation of the lysed blood, followed by the removal of supernatant and plating of the sediment onto the solid media [20,21]. We hypothesized that this solid-medium-based direct cultivation method, applied in combination with MALDI-TOF MS may enable faster bacterial identification than automated BC systems. Furthermore, this study aimed to investigate the use of the early available colonies for antimicrobial susceptibility testing (AST).

Materials and methods

Bacterial strains and preparation of spiked blood samples

Seven reference strains of different bacterial species—*Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Streptococcus pneumoniae* (ATCC 49619) and *Haemophilus influenzae* (NCTC 8468)—were cultured on Columbia blood agar (chocolate agar for *H. influenzae*) for 24 h at 36°C. Suspensions with a standardized turbidity of 0.5 McFarland were prepared in 0.85% saline solution (in a preliminary experiment, an expected cell concentration of 0.5 McFarland standard suspension was determined in triplicate for each strain by serial dilution and colony count of plated suspensions). From 0.5 McFarland suspensions, dilution series were prepared and individually calculated volumes (89–350 µL, based on the results of the preliminary experiment) of the appropriate dilution of each strain were added to 10 mL human whole blood to produce final concentrations of 100, 10 and 1 CFU/mL in blood. The real final inoculum size of each suspension used was verified by plating and incubating on tryptic soy agar, Columbia blood agar (for *Streptococcus pneumoniae*) or chocolate agar (for *H. influenzae*).

Human whole blood was obtained from healthy volunteer donors not receiving any systemic medication. The volunteers provided written informed consent before blood donation. Blood was collected in sterile bags (Leukotrap® WB, Pall Medical, Port Washington, NY, USA).

Sample processing and incubation

Ten millilitres of inoculated blood was aseptically introduced into an Isolator 10 tube (Wampole Laboratories, Princeton, NJ, USA) containing saponin for blood cell lysis and sodium polyanethol sulphonate as an anticoagulant by piercing the rubber septum with a sterile needle. Immediately after adding the spiked blood sample, tubes were gently inverted several times and centrifuged at 3000 *g* for 30 min. After removal of supernatant, 1.5 mL sediment was vortexed and evenly distributed onto five pre-warmed 150-mm Columbia blood agar plates (chocolate agar for *H. influenzae*). All plates were incubated at 36°C in air with 5% CO_2. Growth was observed hourly and microcolonies were subjected to MALDI-TOF MS for identification and to Vitek 2 for AST as soon as it was deemed possible. Incubation of agar plates was continued up to 24 h and both identification and AST were performed from mature colonies as control.

For comparison, seeded blood was inoculated into an aerobic blood culture bottle (BACTEC™ Plus Aerobic/F; BD Diagnostics, Heidelberg, Germany) and incubated in an automated BC system (BACTEC™ 9240; BD Diagnostics). Time to positivity (i.e. growth detection) was automatically recorded.

All experiments were performed in triplicate on different days and median values were calculated for analysis.

MALDI-TOF MS identification

MALDI-TOF MS using intact cell procedure [11] was performed at the time point at which microcolonies became visible and it was considered after visual inspection that the colony material could be sufficient for investigation. Spectra were acquired using the Microflex LT system (Bruker Daltonics, Bremen, Germany) and analysed by MALDI Biotyper 3.1 (Bruker Daltonics) software. MALDI-TOF MS analysis was performed in triplicate, with tests performed simultaneously on the same target slide. Criteria for successful identification were fulfilled if the score of at least one from three spots was $\geq$2.0 (high confidence identification); however, the time point of achievement of low confidence identification (score $\geq$1.7) as well as achievement of the modified threshold (score $\geq$1.5 and first three identical propositions) were also recorded. The experiment was stopped when intact cell identification was successful. If not, intact cell MALDI-TOF MS was repeated hourly until successful identification (score $\geq$2.0). Also, in the case of failed intact cell identification, it was attempted to increase the score at the same time point by (a) giving additional manual laser shots on the same spots, (b) repeating MALDI-TOF MS analysis using the short on-plate extraction procedure [22], and (c) giving additional manual shots if MALDI-TOF MS with on-plate extraction also failed. For control, intact cell MALDI-TOF MS was also performed at 24 h.

Antimicrobial susceptibility testing

During the hourly inspection of plates, the first time point was determined at which the growth appeared sufficient for preparation of 0.5 McFarland suspension to be used for AST by