Journal of Microbiological Methods 148 (2018) 40–45

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

An improved in-house lysis-filtration protocol for bacterial identification from positive blood culture bottles with high identification rates by MALDI-TOF MS

Sachio Tsuchida[a,b], Syota Murata[b], Akiko Miyabe[b], Mamoru Satoh[a], Masaki Takiwaki[a], Kazuyuki Matsushita[b,c], Fumio Nomura[a,c,*]

[a] Division of Clinical Mass Spectrometry, Chiba University Hospital, Chiba, Japan
[b] Department of Clinical Laboratory, Chiba University Hospital, Chiba, Japan
[c] Division of Clinical Genetics, Chiba University Hospital, Chiba, Japan

ARTICLE INFO

Keywords:
Bacterial identification
Blood culture
Matrix-assisted laser desorption ionization
time-of-flight mass spectrometry

ABSTRACT

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is now a well-established method for identification of microorganisms from positive blood cultures. Pretreatments to effectively remove non-bacterial proteins are a prerequisite for successful identification, and a variety of protocols have been reported. Although commercially available kits, mainly the Sepsityper Kit, are increasingly used, the identification rates reported often are not satisfactory, particularly for Gram-positive isolates. We developed a new, in-house lysis-filtration protocol and prospectively evaluated its performance compared to the Sepsityper kit. The in-house protocol consists of three simple steps: lysis by ammonium chloride, aspiration with a syringe fitted with a 0.45-μm membrane, and centrifugation to collect microbes. The novel protocol requires only 20 min.

Performance of the in-house protocol was evaluated using a total of 117 monomicrobial cases of positive blood culture. Medium from blood culture bottles was pretreated by the in-house protocol or the commercial kit, and isolated cells were subjected to direct identification by mass spectrometry fingerprinting in parallel with conventional subculturing for reference identification. The overall MALDI-TOF MS-based identification rates with score > 1.7 and > 2.0 obtained using the in-house protocol were 99.2% and 85.5%, respectively, whereas those obtained using the Sepsityper Kit were 85.4% and 61.5%, respectively. For Gram-positive cases, the in-house protocol yielded scores > 1.7 and > 2.0 at 98.5% and 76.1%, respectively, whereas the commercial kit yielded these scores at 76.1% and 43.3%, respectively. Although these are preliminary results, these values suggest that this easy lysis-filtration protocol deserves assessment in a larger-scale test.

1. Introduction

Rapid and accurate identification of microorganisms is a critical function of clinical microbiology laboratories, facilitating appropriate patient care and infection control. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a quick and reliable method for identification of microorganisms; the role of this technique is rapidly growing in clinical diagnostic microbiology (Neville et al., 2011; Clark et al., 2013; Nomura, 2015; Angeletti, 2017).

Most testing based on MALDI-TOF MS is applied to bacterial colonies grown on agar plates, whereas direct analysis of clinical specimens without need for prior culturing or subcultures increases the usefulness of this technology. Urine is a candidate matrix for direct bacterial identification (Íñigo et al., 2016). Cerebro-spinal fluid (CSF) is another good target matrix; we previously reported that MALDI-TOF MS can provide rapid identification of bacteria from CSF, which can enable early and appropriate treatment (Segawa et al., 2014).

Blood stream infections have a high mortality rate; prompt identification of the causative organism is essential for early initiation of appropriate antimicrobial therapy (Angus and Wax, 2001; Seifert, 2009). It has been reported that each hour of delay in adequate antibiotic treatment for sepsis-related hypotensive patients decreases the chance of survival by 7.6% (Kumar et al., 2006).

Direct identification of bacteria from blood culture bottles is a

Abbreviations: MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MS, Mass spectrometry

* Corresponding author at: Division of Clinical Mass Spectrometry, Chiba University Hospital, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
E-mail address: fnomura@faculty.chiba-u.jp (F. Nomura).

https://doi.org/10.1016/j.mimet.2018.03.014
Received 9 March 2018; Accepted 26 March 2018
Available online 31 March 2018

promising application of MALDI-TOF MS, as has been noted in several reviews (Faron et al., 2017; Florio et al., 2017; Angeletti, 2017). Since blood culture bottle media contain a variety of nonbacterial proteins that may interfere with the interpretation of bacterial proteome profiles, pretreatment to effectively remove host proteins and blood cells while also concentrating the microbes is a key step for successful identification. A variety of laboratory-developed or commercially available protocols for purification of bacterial pellets have been reported (as reviewed by Florio et al. (2017) and Scott et al. (2016)). The following are representative examples of the laboratory-developed test protocols: 1) stepwise sedimentation of blood cells and microorganisms (Ferreira et al., 2011), 2) low-speed centrifugation to remove blood cells, followed by an additional lysis procedure (Prod'hom et al., 2010; Monteiro et al., 2015) and 3) removal of blood cells by serum separator tube (Moussaoui et al., 2010). The Sepsityper kit (Bruker Daltonics, Lepzig, Germany) (Buchan et al., 2012) and the Vitek MS blood culture kit (bioMerieux, Inc., Marcy l'Etoile. France) (Fothergill et al., 2013) are commercial purification kits. The overall concordance of the results obtained with laboratory-developed test protocols and commercial kits with those obtained by the conventional direct colony methods ranges from 84 to 99% for Gram-negative bacterial identification and is approximately 80% for Gram-positive cases (Faron et al., 2017). A meta-analysis of the performance of the Sepsityper kit indicated that this kit provided reliable identification, on the species level, of the microorganisms from 80% of 3320 positive blood culture bottles (Morgenthaler and Kostrzewa, 2015). Again, Gram-negative bacteria were identified at a higher frequency (90%) than were Gram-positive pathogens (60%). In contrast to the case with the Sepsityper kit, only a limited number of reports have been published regarding the Vitek MS blood culture kit (Fothergill et al., 2013; Abdallah et al., 2015). The original Vitek MS blood culture kit is a lysis-filtration method that is performed using a manifold attached to a vacuum source. Since the Vitek MS blood culture kit is not readily accessible for non-Vitek MS users in Japan, we have developed an in-house lysis filtration-method. We have additionally prospectively evaluated the performance of this technique (compared to that of the Sepsityper kit) when used for the preparation of samples from positive blood culture bottles for bacterial identification by MALDI-TOF MS.

2. Materials and methods

2.1. Samples and study design

The clinical evaluation was conducted during working hours from June 2016 to June 2017 at the Chiba University Hospital, Chiba, Japan. During this study period, a total of 120 blood culture bottles from 120 patients were flagged as positive. BD BACTEC™ Plus Aerobic medium in glass culture vials (BD, Franklin Lakes, NJ, USA) and the BACTEC™ FX incubation system (BD, Franklin Lakes, NJ, USA) were used. A portion of positive culture bottles were Gram stained followed by subculturing. The subcultures were conducted on 5% sheep blood agar (SBA) and chocolate agar. Eosin methylene blue and anaerobic SBA were used for Gram-negative bacteria and anaerobic bottle subcultures, respectively. The blood cultures were held at room temperature following removal from the blood culture instrument and were analyzed within 8 h of culture positivity identification. None of the positive cultures failed to grow on subculturing, and 3 were polymicrobial. The remaining 117 monomicrobial cases were included in the comparative analysis between the in-house lysis and filtration protocol and the commercial Sepsityper Kit. Positive monomicrobial isolates were subjected in parallel to direct identification by mass spectrometry fingerprinting and conventional subculturing for reference identification.

2.2. Conventional identification

All solid media were incubated at 37 °C in 5% CO_2 for 18–24 h. Each clinical specimen was plated on an appropriate solid medium (chocolate agar or trypticase soy agar II with 5% sheep blood, Nippon Becton Dickinson, Tokyo, Japan) depending on the sample and in accordance with our laboratory recommendations. Following Gram staining and determination of catalase and oxidase activities, the isolates were identified by three different phenotypic tests, including the Microscan Walkaway system (Siemens Healthcare Diagnostics, Deerfield, IL, USA), the BD Phoenix system (BD Diagnostics Systems, Sparks, MD, USA), and the API system (Sysmex Biomerieux, Lyon, France). The respective manufacturer's instructions for each system were followed.

2.3. Sample pretreatment methods for direct MALDI-TOF MS analysis

2.3.1. In-house lysis and filtration protocol

A 2-mL blood-culture fluid sample was added to a reaction tube containing 100 µL of ammonium chloride lysis buffer (BD PharmLyse™; BD Biosciences, San Jose, CA, USA) and 900 µL of phosphate-buffered saline (PBS). The mixture was vortexed at high speed for 10 s and the 3-mL mixture then was manually drawn into a 10-mL syringe fitted with a 0.45-µm membrane (eQuality monitor; Pall Life Sciences, Port Washington, NY, USA). This procedure was completed within (on average) 2 min.

Subsequently, each membrane was manually washed with 5 mL of distilled water (DW) delivered using a 10-mL syringe with a 0.45-µm membrane quality monitor. This procedure was completed within (on average) 3 min.

Next, the membrane was placed into a 50-mL tube containing 1 mL of DW; the tube was vortexed at high speed for 30 s to wash the membrane, and 1 mL of the resulting liquid was transferred to an Eppendorf Safe-Lock Tube (Eppendorf Japan, Tokyo, Japan). The Eppendorf tube then was centrifuged at 13,000 ×*g* for 3 min.

The pellet formed at the bottom of the tube was recovered by scraping using a sterile toothpick. For safety, the harvest strokes were made in a direction away from the face. The pellet adhering to the toothpick was directly transferred onto the MALDI target plate spotting surface in an up-and-down rather than a sliding motion. Immediately, 1 µL of 70% formic acid was spotted onto the pellet resting on the MALDI target plate. Two spots on the MALDI target plate were prepared for each sample. After drying, 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA) was applied to each spot, and the spot then was allowed to air dry. An outline of this in-house lysis filtration method is illustrated in Fig. 1.

2.3.2. MALDI Sepsityper® kit

One milliliter of the medium from each positive blood culture bottle was combined with 200 µL of the Sepsityper® lysis reagent (Bruker Daltonics). The mixture was vortexed for 10 s and then held at room temperature for 5 min before being centrifuged for 1 min at 13,000 ×*g*. Following centrifugation, the supernatant was decanted; the pellet was re-suspended in 1 mL of Sepsityper® Washing Buffer (Bruker Daltonics) and centrifuged again for 1 min at 13,000 ×*g*. The supernatant was decanted and the pellet was re-suspended in 300 µL MALDI-grade water and 900 µL 100% ethanol followed by centrifugation for 2 min at 13,000 ×*g*. The supernatant then was decanted and each sample re-centrifuged for another 2 min at 13,000 ×*g*. Any residual ethanol then was removed and the pellet was dried for 10 min at room temperature. Extractions were performed according to the manufacturer's recommendations. In brief, 30 µL of 70% formic acid and 30 µL of 100% acetonitrile were added to the tube; the pellet was thoroughly re-suspended; and the sample was centrifuged for 2 min at 13,000 ×*g*. One microliter of the supernatant then was transferred to a sample spot on a MALDI target plate and the spot was allowed to air dry. Two spots on the MALDI target plate were used for each sample. One microliter of α-CHCA matrix solution (Bruker Daltonics) was added to each spotted sample and the spots were allowed to air dry.