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Optimal turnaround time for direct identification of microorganisms by mass spectrometry in blood culture

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ABSTRACT

Introduction: During the past few years, several studies describing direct identification of bacteria from blood culture using mass spectrometry have been published.
These methods cannot, however, be easily integrated into a common laboratory workflow because of the high hands-on time they require. In this paper, we propose a new method of identification with a short hands-on time and a turnaround time shorter than 15 min.
Materials and methods: Positive blood bottles were homogenised and 600 μL of blood were transferred to an Eppendorf tube where 600 μL of lysis buffer were added. After homogenisation, a centrifugation step of 4 min at 10,500*g* was performed and the supernatant was discarded. The pellet was then washed and loaded in quadruplicate into wells of a Vitek® MS-DS plate. Each well was covered with a saturated matrix solution and a MALDI-TOF mass spectrometry analysis was performed. Species were identified using the software Myla 3.2.0-2.
Results: We analysed 266 positive blood culture bottles. A microorganism grew in 261 cultures, while five bottles remained sterile after 48 h of incubation in subculture. Our method reaches a probability of detection at the species level of 77.8% (203/261) with a positive predictive value of 99.5% (202/203).
Conclusion: We developed a new method for the identification of microorganisms using mass spectrometry, directly performed from a positive blood culture. This method has short hands-on time and turnaround time and can easily take place in the workflow of a laboratory, with comparable results in performance with other methods reported in the literature.

1. Introduction

Bloodstream infections are severe pathologies associated with high rates of morbidity and mortality in hospitals (Wisplinghoff et al., 2004). Blood culture is the gold standard for detecting and identifying microorganisms causing sepsis (Dellinger et al., 2013) and rapid identification of pathogenic organisms from blood cultures enables early initiation of antibiotic treatment in patients with bacteraemia (Seifert, 2009).

Introduction of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in microbiology laboratories allowed a significant time saving for identification of microorganisms after using solid phase media. This technology generates characteristic mass spectra that are unique signatures for each microorganism. The major limitation is the amount of bacteria present in the samples and the limit of detection of current MALDI-TOF protocols (Croxatto et al., 2012).

Conventionally, identification using MALDI-TOF MS is performed on colonies grown on agar plate after 18–48 h of incubation. During the past few years, several studies related to direct identification of bacteria from blood culture using mass spectrometry have been published (Bessède et al., 2014; Verroken et al., 2015; Machen et al., 2014; Jakovljev and Bergh, 2015; Frederic et al., 2015; Monteiro et al., 2015; Rodríguez-Sánchez et al., 2013; Lagacé-Wiens et al., 2012). The reported methods were based on identification after a short period of subculture from 3 to 5 h after the bottle became positive (Bessède et al., 2014; Verroken et al., 2015); or an aliquot directly taken from blood culture bottle when it becomes positive (Machen et al., 2014; Jakovljev and Bergh, 2015; Frederic et al., 2015; Monteiro et al., 2015; Rodríguez-Sánchez et al., 2013; Lagacé-Wiens et al., 2012).

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These methods cannot, however, be easily integrated into a common laboratory workflow because of the variety of the internal organization of microbiology laboratories (working 24 h/24 or not, dedicated staff, etc.) and the high hands-on time they may require.

In this paper, we propose a new method of identification, directly performed from blood culture, with a short hands-on time and a turn-around time smaller than 15 min once the bottle became positive and the moment we have identified the pathogen. The results were compared with the classical process used in our laboratory.

2. Materials and methods

2.1. Blood culture collection and classical process

From August 2015 to December 2015, blood cultures bottles from adult patients were collected at the Laboratory of Clinical Biology of the Cliniques du Sud Luxembourg in Arlon, Belgium. They were systematically collected in BacT/Alert® FA Plus aerobic and BacT/Alert® SN anaerobic bottles (Biomérieux®, Marcy-l'Etoile, France). After the reception and encoding at the laboratory, the bottles were immediately incubated at 37 °C in the BacT/Alert 3D system (Biomérieux®, Marcy-l'Etoile, France) for a maximum of 5 days or until they became positive for bacterial growth.

When the blood culture was detected positive by the system, the samples were immediately processed for Gram staining and cultivated for strain identification by MALDI-TOF MS after at least 18 h of incubation. The media used for the subcultures of aerobic and anaerobic bottles were Columbia Agar with 5% sheep blood (Biomérieux®, Marcy-l'Etoile, France) with the addition of a tablet of Diatabs™ Factor V (Rosco Diagnostica A/S, Taastrup, Denmark), and Mac Conckey Agar/Columbia CNA Agar with 5% sheep blood (Biomérieux®, Marcy-l'Etoile, France) with the addition of a tablet of Diatabs™ Optochin 10 μg (Rosco Diagnostica A/S, Taastrup, Denmark) on CNA agar, respectively. Both were incubated for 18–48 h at 35 °C in CO_2 atmosphere. A sample from anaerobic bottle was also incubated on Schaedler Agar/Schaedler KV Selective Agar (Oxoid Deutschland GmhB, Wesel, Germany) for 48 h in anaerobic atmosphere. This classical identification methodology, routinely used in our laboratory, will be considered as control for our rapid identification test.

2.2. Blood culture direct processing

In the present study, the identification was directly performed on an aliquot taken from positive blood culture bottles. Positive blood bottles were homogenised and 600 μL of blood was transferred to a 1.5 mL Eppendorf tube. A volume of 600 μL of lysis buffer from Vitek® MS Blood Culture Kit (Biomérieux®, Marcy-l'Etoile, France) was added in the tube and homogenized for 5 s by vortexing. A centrifugation step of 4 min at 10,500g was performed and the supernatant was discarded. The pellet was washed with the wash buffer from Vitek® MS Blood Culture Kit. A sample of the bacterial pellet was taken with a Puritan® PurSwab 3″ stick (Puritan Medical Products Company LLC, Guilford, Maine, USA) and loaded in quadruplicate into wells of a Vitek® MS-DS plate (Biomérieux®, Marcy-l'Etoile, France). Each well was covered with 1 μL of saturated matrix solution of Vitek® MS-CHCA (Biomérieux®, Marcy-l'Etoile, France) and a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry analysis was performed on a Vitek® MS (Biomérieux®, Marcy-l'Etoile, France). Spectra were acquired by the standard recommended method. Species were identified using the software Myla 3.2.0-2 (Biomérieux®, Marcy-l'Etoile, France). After comparison with its database, the software ranked a matching probability for identification. For identification of pathogens from agar media, values >95.0% are required as cut-off for secure identification at the species level. Figs. 1 and 2 illustrate the handling protocol.

3. Results

During the period of the study, we analysed 266 positive blood culture bottles: 157 from aerobic and 109 from anaerobic bottles. A total of 279 microorganisms grew in 261 cultures, while five bottles remained sterile after 48 h of incubation in subculture. Identification of a microorganism was possible in 214 of the 261 positive bottles (82.0%) through direct identification with a matching of 97.7% with the culture (209/214). These results correspond with a detection of 78.5% for the positive anaerobic bottles (84/107) and 84.4% for the positive aerobic bottles (130/154). Two pathogenic organisms were detected in 6.9% of the cultures (18/261). The direct identification of one of the microorganisms was possible for 61.1% in these cases (11/18).

3.1. Identification score

A number of 203 on 261 bottles (77.8%) obtained an identification score >95.0%: the correlation with the culture reached 99.5% (202/203). All identification scores <60.0% are considered as "no identified", as notified by the manufacturer: this represents 18.0% of the positive bottles (47/261). However, it is worth noting that the 5 negative cultures also showed a negative result for the rapid identification. When an *Enterobacter cloacae* was brought out, the analyzer could not make the difference at the species level between *Enterobacter cloacae* and *Enterobacter asburiae*: the device reported an identification score of 50% - 50%. This also happened with our reference protocol after subculture. The operator must manually select the species. This choice has no clinical impact and *Enterobacter cloacae* is always selected in the routine protocol of our laboratory. The correlation with culture for these 3 cases (1.2%) was 100%. The remaining 3.1% (8/261) presented an identification score ranging from 60.0 to 94.9%, with a matching of 50.0% (4/8) with the culture.

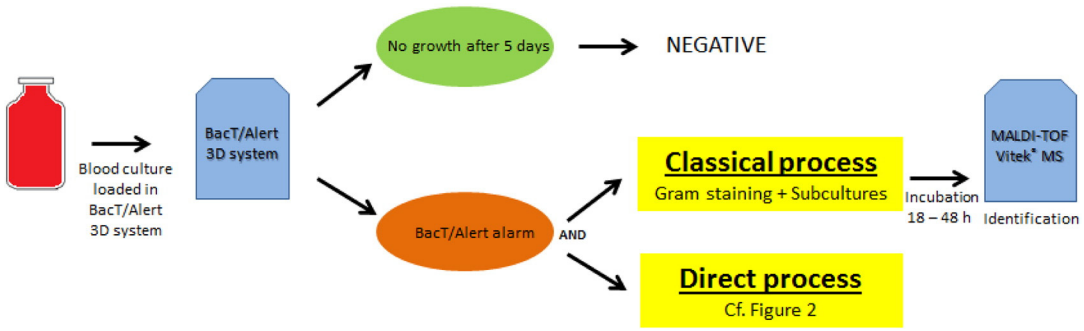

Fig. 1. Management of blood cultures bottles.