Turnaround time of positive blood cultures after the introduction of matrix-assisted laser desorption-ionization time-of-flight mass spectrometry

Silvia Angeletti, Giordano Dicuonzo, Alfio D'Agostino, Alessandra Avola, Francesca Crea, Carlo Palazzo, Etleva Dedej, Lucia De Florio

Clinical Pathology and Microbiology Laboratory, University Hospital Campus Bio-Medico of Rome, Italy

—————————————— SUMMARY ——————————————

A comparative evaluation of the turnaround time (TAT) of positive blood culture before and after matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) introduction in the laboratory routine was performed.

A total of 643 positive blood cultures, of which 310 before and 333 after MALDI-TOF technique introduction, were collected.

In the post MALDI-TOF period, blood culture median TAT decreased from 73.53 hours to 71.73 for Gram-positive, from 64.09 hours to 63.59 for Gram-negative and from 115.7 hours to 47.62 for anaerobes. MALDI-TOF significantly decreased the TAT of anaerobes, for which antimicrobial susceptibility test is not routinely performed. Furthermore, the major advantage of MALDI-TOF introduction was the decrease of the time for pathogen identification (T_{ID}) independently from the species with an improvement of 93% for Gram-positive, 86% for Gram-negative and 95% for anaerobes.

In addition, high species-level identification rates and cost savings than conventional methods were achieved after MALDI-TOF introduction.

KEY WORDS: Turnaround time (TAT), Blood culture, MALDI-TOF.

INTRODUCTION

Sepsis is a potentially fatal condition that can evolve from localized bacterial infections, and represents the tenth cause of death in the USA. In Europe, sepsis occurs in more than 35% of the patients in the intensive care unit.

Nearly 9% of patients with sepsis progress into severe sepsis and nearly 3% have a septic shock which leads to multi-organ failure and death.

More than 50% of patients who experience septic shock do not survive (Dellinger *et al.*, 2013; Levy *et al.*, 2003; Angus *et al.*, 2001). If the mortality associated with sepsis is approximately 28%, it rises to 40-60% in septic shock, despite adequate treatment.

The rapid recognition and treatment of sepsis is a fundamental requirement to reduce both the mortality associated with this condition and hospitalization with related costs (Dellinger *et al.*, 2013; Levy *et al.*, 2003; Angus *et al.*, 2001). The gold standard for detection of bloodstream infections is blood culture.

The time required for a positive blood culture result depends on the incubation time required for the culture to turn positive and the subsequent biochemical identification, along with antibiotic sensitivity test, both of which usually take 48 h (Cherkaoui *et al.*, 2010). In

Corresponding author
Silvia Angeletti
University Hospital Campus Bio-Medico of Rome
Via Alvaro del Portillo, 200 - 00128 Rome, Italy
E-mail: s.angeletti@unicampus.it

recent years, automated blood culture systems have been improved to reduce the incubation time necessary for a positive blood culture identification.

Furthermore, new molecular approaches such as PCR, fluorescence in situ hybridization and DNA sequencing have been applied to expedite pathogen identification (Frye *et al.*, 2012; Bauer *et al.*, 2010; Kempf *et al.*, 2000; Jordan *et al.*, 2009; Angeletti *et al.*, 2013). These molecular techniques have some limitations, particularly the high cost and the need for expert laboratory staff.

Recently, a new method, MALDI-TOF mass spectrometry (MALDI-TOF MS), for the rapid identification of most pathogenic bacteria has been described (Stevenson *et al.*, 2010; Ferroni *et al.*, 2010; Christner *et al.*, 2010).

Turn around time (TAT) is a key indicator of clinical laboratory performance and has a major impact on the diagnosis and management of septic patients and on the length of stay in the intensive care unit (Howanitz and Howanitz, 2001; Martinez *et al.*, 2014; Rodriguez-Sanchez *et al.*, 2014).

Total TAT is the entire time interval from the test order to the clinician's decision based on the blood culture results (Valenstein, 1996; Kilgore *et al.*, 1998).

Time from order to specimen collection as well as the time for transport to the laboratory are not under control of the laboratory staff so that it is preferable to use intra-laboratory TAT as a quality indicator of laboratory performance. The management of septic patients can be improved by the administration of the appropriate treatment without delay, thus TAT for blood culture processing represents an important factor influencing patient treatment and recovery (Retamar *et al.*, 2012; Lever and Mackenzie, 2010; Zubert *et al.*, 2007).

The aim of the present study was to evaluate the TAT for pathogen identification in positive blood culture after the introduction of a rapid system for bacterial identification based on MALDI-TOF technology and to compare this finding to the classical work flow of blood culture under traditional standard conditions. Furthermore, as a second aim, the global TAT from the check-in of the blood culture set to the final report was evaluated.

MATERIALS AND METHODS

Blood culture collection and processing
From January 2012 to December 2013, 3086 blood culture sets were collected at the Laboratory of Clinical Pathology and Microbiology of the University Hospital Campus Bio-Medico of Rome.

Blood specimens from adult patients were systematically collected in BACTEC bottles containing anaerobic (BACTEC anaerobic (BAA) or aerobic (BACTEC aerobic (BA)) broth and resins. Bottles containing 25-30 mL of whole blood were incubated in BACTEC FX instrument (Becton Dickinson, Meylan, France) immediately upon arrival in the laboratory.

All blood cultures were received in an integrated laboratory reception that processed samples from 7:00 to 20:00, 7 days a week. After the check-in blood culture sets were continuously incubated in the Bactec FX (Becton Dickinson, Meylan, France) for a maximum of 5 days or until they became positive for bacterial growth. BC samples that turned positive during regular duty hours (09:00-20:00) were immediately processed for Gram staining and routinely cultivated for subsequent biochemical identification, a process that usually began the following day, as well as prepared for direct MALDI-TOF processing.

Intra-laboratory TAT was calculated as the time from the check-in to the final report with identification and antibiotic susceptibility test results.

Separation and protein extraction for blood culture direct processing in the MALDI-TOF system: rapid microbial identification in the year 2013 (post-MALDI-TOF period
Approximately 8.0 mL of blood were recovered from positive BC vials, transferred to a 15 ml conic tube and centrifuged at 600 g for 10 minutes. After recovery, 1.5 mL of supernatant was collected in a new 1.5 ml tube for the second centrifugation step at 13000 rpm for 10 minutes and the bacterial pellet collected.

The bacterial pellet was treated with the standard ethanol/formic acid protein extraction protocol for MALDI-TOF MS identification (Mellmann *et al.*, 2009). One microliter of protein extract was then loaded in duplicate into

wells of a polished steel TF MTP 384 target plate (Bruker Daltonics, Germany), covered with 1 µL of a saturated solution of a-cyano-4-hydroxycinnamic acid/MALDI-TOF matrix in 50% (v/v) acetonitrile/2.5% (v/v) trifluoroacetic acid (Sigma-Aldrich, Milan, Italy) as an electron donor. MALDI-TOF was performed on a Microflex LT controlled by FlexControl version 3.4 software (Bruker). Spectra were acquired by the standard recommended proprietary method utilizing the Biotyper preprocessing standard method and the Biotyper Main-Spectrum (MSP) identification standard method (2,000 to 20,000 Da; linear positive method; laser frequency of 60 Hz). Species were identified using the MALDI Biotyper 3.1 (Bruker) and its standard database (Bruker Taxonomy database version 3.3.1).

The software employed, Bruker Biotyper 3.1 (Bruker Daltonics GmbH, Bremen, Germany), automatically acquired spectra with fuzzy control of the laser intensity and analyzed them by standard pattern matching against the spectra of 5627 species used as reference data. After comparing the unknown spectra with all reference spectra in the database, the log scores were ranked. Values of >1.9 were required for secure identification at the species level, and values between 1.9 and 1.7 were required for secure identification at the genus level. Identification of microorganisms using the MALDI Biotyper 3.1 was possible within 1 hour from blood culture positivity.

Bacterial identification in positive blood culture in the year 2012 (pre-MALDI-TOF period
For Gram-negative bacteria a rapid and direct inoculation system was used in the year 2012 (pre-MALDI-TOF period), as previously described (Gherardi *et al.*, 2012).
For Gram-positive and anaerobe microorganisms identification in the year 2012 (pre-MALDI-TOF period), the standard method by Vitek-2 Compact was used, as previously described (22).

Antimicrobial susceptibility test (AST) by Vitek-2 Compact (pre and post-MALDI-TOF periods)
For Vitek-2 Compact, 145 and 280 µL inoculum was used for Gram-negative and Gram-positive,

respectively. The cards AST-P632 and AST-P586 for staphylococci and enterococci, respectively, and AST-202 and AST-204 for Gram-negative rods were used for AST according to the manufacturer's instructions. The Vitek-2 AST cards were logged and loaded into the Vitek-2 Compact system. The Vitek-2 Compact system automatically reported the results through software 05.01. AST was not performed in case of anaerobes microorganism identification, because antibiotic therapy is empirical (Cohen-Poradosu and Kasper, 2015).

Intra-laboratory TAT
TAT for positive blood cultures processing and report (Final TAT, T_F) can be divided in two components T_1, always corresponding to the time to positivity (T_P) necessary for the microorganism growth during BACTEC FX incubation, and T_2, from bottle positivity to final report, including a time for pathogen identification (T_{ID}) and a time for antimicrobial susceptibility test (T_{AST}). TATs were calculated and documented through the aid of the laboratory software package (MedLis) interfaced with MedArchiver, the computer platform for patient record management.

RESULTS

During the study period 643 positive blood cultures, of which 310 before and 333 after MALDI-TOF technique introduction into clinical laboratory routine, were collected. 294/333 (88%) blood cultures processed by MALDI-TOF were subjected to a rapid protocol for microbial identification. The number of positive blood cultures and the time to positive blood culture results for each class of microorganisms collected during the pre and post-MALDI-TOF periods are reported in Table 1.
MALDI-TOF rapid protocol was performed in 294/333 positive blood cultures and a correct identification was achieved in 243/294 (83%) positive blood cultures (Table 2). MALDI-TOF did not give any reliable identification in 51/294 (17%) positive blood cultures (Table 2).
As reported in table 2, the number of blood cultures identified or not identified with the rapid protocol by the MALDI-TOF technique has

TABLE 1 - *Number of positive Blood Culture (BC) in the years 2012 (pre-MALDI-TOF) and 2013 (post-MALDI-TOF) and median time (in hours) to positive results for the different classes of bacteria.*

	N° of Positive BC Year 2012	N° of Positive BC Year 2013	Time to positive result (hours)
Gram-positive	132	161	20.53
Gram-negative	99	140	13.49
Yeast	31	16	33.47
Polimicrobial	36	-	nd
Anaerobe	12	16	44.22
Total	310	333	-

TABLE 2 - *Performance of MALDI-TOF rapid protocol in positive blood culture microorganisms identification (ID).*

	N° isolates	N° MALDI-TOF ID performed	N° Reliable ID (score ≥2) (%)	N° not reliable ID (score ≤1.7) (%)
Gram-positive	161	134	104 (78%)	30 (22%)
Gram-negative	140	134	126 (94%)	8 (6%)
Yeast	16	10	2 (20%)	8 (80%)
Polimicrobial	70	nd	nd	nd
Anaerobe	16	16	11 (69%)	5 (31%)
Total	403	294	243 (83%)	51 (17%)

been given for each class of microorganism. Gram-positive cultures were identified by the rapid protocol in 104/134 (78%). MALDI-TOF failed direct identification of 30/134 (22%) gram-positive bacteria. Gram-negative cultures were identified in 126/134 (94%) cases and anaerobes in 11/16 (69%) cases. Gram-negative and anaerobe microorganisms identification failed in 8/134 (6%) and 5/16 (31%), respectively. Sixteen blood cultures were positive for yeast, 10/16 were processed by MALDI-TOF rapid method but only 2/10 (20%) gave a correct identification as that achieved traditionally.

The two different components (T_{ID}, T_{AST}) of the final TAT (T_F) recorded in the year 2012, pre-MALDI-TOF period and in the year 2013, post-MALDI-TOF period, are reported in Table 3 and schematized in Figure 1. Table 4 shows a comparison of the median T_F for Gram-positive, Gram-negative and anaerobes recorded in the year 2012 and 2013.

In the year 2013 (post MALDI-TOF period), Gram-positive and Gram-negative T_F did not decrease substantially with the introduction of the MALDI-TOF technique (73.53 hours vs 71.73 for Gram-positive and 64.09 hours vs 63.59 for Gram-negatives) (Table 4).

In the year 2012, the component T_2 of the final

TABLE 3 - *Median Time and standard deviation for pathogen identification (T_{ID}) and for antimicrobial susceptibility test (T_{AST}) registered in the year 2012 (pre-MALDI-TOF) and 2013 (post-MALDI-TOF).*

	Gram-positive	Gram-negative	Anaerobes
T_{ID} 2012 year SD for mean	53.0 30.9	27.2 6.3	71.5 146.6
T_{ID} 2013 year SD for mean	3.7 2.8	3.8 1.9	3.4 1.9
T_{AST} 2012 year SD for mean	53.0 30.9	50.6 19.9	-
T_{AST} 2013 year SD for mean	51.2 20.5	50.1 22.9	-

TABLE 4 - *Median Final TAT in hours for positive blood culture registered in the year 2012 (pre-MALDI-TOF) and 2013 (post MALDI-TOF).*

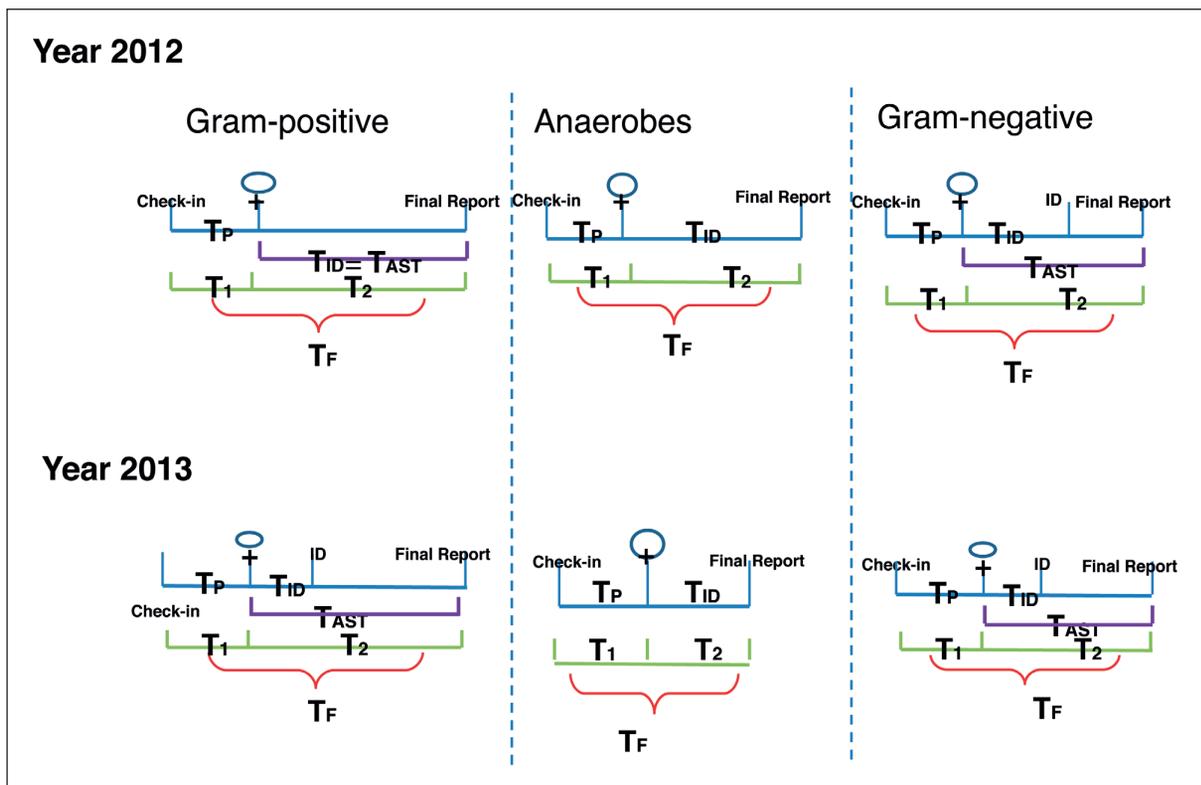|	Final TAT Gram-positive	Final TAT Gram-negative	Final TAT Anaerobes
Year 2012 (Pre-MALDI-TOF)	73.53	64.09	115.7
Year 2013 (Post-MALDI-TOF)	71.73	63.59	47.62

FIGURE 1 - *Schematic representation of the final TAT (TF) and its component T1 (corresponding to the time to positivity TP) and T2, including a time for pathogen identification (TID) and a time for antimicrobial susceptibility test (TAST), in Gram-positive, Gram-negative and anaerobe blood culture during the years 2012 (upper half of the figure) and 2013 (lower half of the figure).*

TAT for Gram-positive cultures corresponded to T_{ID} as well as to T_{AST} times, while in the year 2013, the median T_{ID} substantially decreased from 53 hours (2012 year) to 3.7 hours (2013 year) with a percentage of improvement of about 93% while T_{AST} remained substantially unchanged (Table 3 and Figure 1). In the year 2012, the T_2 component for Gram-negative microorganisms corresponded to the T_{AST} necessary for the antimicrobial susceptibility test, while T_{ID} was shorter than Gram-positive microorganisms because a rapid phenotypic method was applied (Gherardi *et al.*, 2012). In the year 2013, after MALDI-TOF introduction, the median T_{ID} further decreased from 27.2 to 3.8 hours with a percentage of improvement of about 86%, while T_{AST} remain substantially unchanged as in the case of Gram-positive microorganisms (Table 3 and Figure 1).
In the case of anaerobe bacteria, the T_2 component included only the median T_{ID} that de-

creased from 71.5 hours recorded in the 2012 to 3.4 in the year 2013 after the introduction of the MALDI-TOF technique with a percentage of improvement of about 95% (Table 3 and Figure 1).
The median final TAT (T_F) substantially decreased in the 2013 (post MALDI-TOF period) for anaerobe microorganisms from 115.7 hours to 47.62 the T_2 component being affected only by the T_{ID} time that benefits from the introduction of the MALDI-TOF rapid protocol (Tables 3 and 4).

DISCUSSION

The present study applied and evaluated the MALDI-TOF technique for direct microorganism identification in monomicrobial bloodstream infections.
The TAT for final report production was com-

pared to that recorded in the pre-MALDI-TOF period to assess the advantages of the new procedure.

In addition to the high species-level identification rates achieved using the MALDI-TOF method, time and cost savings were significant compared to conventional methods of bacterial identification, as other authors reported (Mestas *et al.*, 2014; Chen *et al.*, 2013; Martinez *et al.*, 2014). Furthermore, identification of microorganisms was possible within 1 hour from blood culture positivity, even if blood culture processing during routine laboratory work may delay the process to 3-4 hours, as shown in *Table 3*. Compared to the 20-46 hours required to subculture, incubate, and perform confirmatory biochemical tests, the MALDI-TOF method used in this study is a reliable alternative to provide rapid identification directly from positive blood cultures.

In a previous study, we evaluated the diagnostic value and the turnaround time of a real-time polymerase chain reaction with melting analysis in bloodstream infections. A TAT of three hours from the moment of blood culture positivity was achieved by molecular methods (Angeletti *et al.*, 2013), but in our experience with higher costs and the need for skilled and dedicated staff than the MALDI-TOF technique.

Recently, Dixon *et al.* made a systematic review of the literature comparing MALDI-TOF and routine methods for the identification of pathogens in patients with suspected bloodstream infection: MALDI-TOF identification was at least 24 h faster than routine methods and it was also associated with a reduction in hospital costs and length of stay (Dixon *et al.*, 2015). Furthermore, several other studies reported that a reduction in the diagnostic work up with the identification of the causal germ of bloodstream infections allows an earlier and more targeted antibiotic therapy, a shorter hospital stay and a consequent reduction in the cost of clinical assistance to the patient (Leli *et al.*, 2013; Chen *et al.*, 2013; Martinez *et al.*, 2014). Moreover, MALDI-TOF identification performed in our laboratory costs 3.2 euro/identification versus 6.7 euro/identification by routine phenotypic methods.

Data from this study demonstrated that the MALDI-TOF introduction substantially de-

creased T_{ID} independently from the species, even if this improvement was less evident in Gram-negative than Gram-positive microorganisms because a previous rapid protocol for direct Gram-negative processing had been applied since the year 2012, as reported in a previous study (Gherardi *et al.*, 2012). Conversely, in anaerobes the T_{ID} improved by 95% because the rapid protocol by MALDI-TOF bypassed the longer growth time necessary for these microorganism than other species.

On the contrary, the final TAT (T_F) is not influenced by MALDI-TOF introduction, except for anaerobes. In fact, the T_F depends on the time required for AST performance that does not benefit from MALDI-TOF in the case of Gram-positive and Gram-negative microorganisms. For these species the T_F improvement recorded is not significant.

Conversely, in the case of anaerobe microorganisms, for which AST is not routinely performed, the antibiotic therapy being empirical (Cohen-Poradosu and Kasper, 2015), the T_F improvement was about 95%: in fact T_F corresponds to T_{ID}. This improvement substantially reflects the advantages derived from the MALDI-TOF introduction in direct positive blood culture processing.

In conclusion, this study describes a simple rapid method for the direct identification of Gram-positive, Gram-negative and anaerobe pathogens directly from positive blood cultures. The simplicity of the method warrants immediate testing once bottles flag positive, allowing for rapid identification and reporting of significant bloodstream pathogens to clinicians.

MALDI-TOF decreases the time for bacterial identification with a good performance, this method is reliable, rapid and cost saving, but AST reduces the advantages of the earlier identification achieved by MALDI-TOF.

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