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ARTICLE

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Abstract Speeding up the turn-around time of positive blood culture identifications is essential in order to optimize the treatment of septic patients. Several sample preparation techniques have been developed allowing direct matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification of positive blood cultures. Yet, the hands-on time restrains their routine workflow. In this study, we evaluated an approach whereby MALDI-TOF MS identification without any additional steps was carried out on short subcultured colonies from positive blood bottles with the objective of allowing results reporting on the day of positivity detection. Over a 7-month period in 2012, positive blood cultures detected by 9 am with an automated system were inoculated onto a Columbia blood agar and processed after a 5-h incubation on a MALDI-TOF MicroFlex platform (Bruker Daltonik GmbH). Single-spotted colonies were covered with 1 μl formic acid and 1 μl matrix solution. The results were compared to the validated identification techniques. A total of 925 positive blood culture bottles (representing 470 bacteremic episodes) were included. Concordant identification was obtained in 727 (81.1 %) of the 896 monomicrobial blood cultures, with failure being mostly observed with anaerobes and yeasts. In 17 episodes of polymicrobic bacteremia, the identification of one of the two isolates was achieved in 24/29 (82.7 %) positive cultures. Routine implementation of MALDI-TOF MS identification on young positive blood subcultures provides correct results to the clinician in more than 80 % of the bacteremic episodes and allows access to identification results on the day of blood culture positivity detection, potentially accelerating the implementation of targeted clinical treatments.

Introduction

Sepsis is a frequent and severe infection, requiring early, appropriate, and targeted antibiotic treatment to reduce the patient's morbidity and mortality. Speeding up the turn-around time of positive blood culture identification results becoming available to the clinician is, therefore, of major importance [1–3].

Various rapid molecular techniques have been developed in order to allow the identification of pathogens growing from blood cultures within 2 h, but also for the direct detection of pathogens in blood samples without any requirement of culture [4, 5]. Associated with a high rule-in diagnostic value but a suboptimal sensitivity, polymerase chain reaction (PCR)-based pathogen detection is, at this time, only recommended as an addition to conventional culture techniques [5]. Their availability remains, furthermore, restricted to a limited number of laboratories, as they are very costly as well as labor and time demanding.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a new technology for species identification analyzing the protein composition of a bacterial cell. Through the improvement of the technique, MALDI-TOF MS has proved over the recent

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years to be a rapid, accurate, easy-to-use, and inexpensive universal method for the identification of microorganisms [6]. Subsequently, various purification and extraction methods have been developed with MALDI-TOF MS for the direct identification of positive blood cultures, allowing the reporting of species results within 1 h after the detection of blood culture positivity [7–15]. However, direct MALDI-TOF MS identification protocols include several washing and extraction steps, requiring additional hands-on time. When considering their workflow implementation, most authors process the positive blood culture specimens in batches, thereby reducing the major time gain advantage on the reporting of the identification results [9, 10, 16, 17].

In this study, we validated an identification process consisting of the MALDI-TOF MS analysis of positive blood subcultures after a shortened 5-h incubation without any preparation steps. This process required much reduced hands-on time, while also allowing results reporting on the same day of blood culture positivity detection. In a second step, a work scheme integrating this process into the identification of positive blood cultures in daily routine practice was defined.

Materials and methods

Positive blood cultures

The study was conducted at the Cliniques universitaires Saint-Luc (UCL), Brussels, Belgium, a 964-bed tertiary hospital. Positivity of all patients' blood culture bottles (BACTEC Plus Aerobic/F, Plus Anaerobic/F, and Peds Plus/F Medium, Becton Dickinson, Franklin Lakes, NJ, USA) was detected with the BACTEC FX automated system (Becton Dickinson, Franklin Lakes, NJ, USA). Specific blood culture bottles for the recovery of yeast were not used in our hospital.

During a 7-month period in 2012, all overnight and early morning weekdays (from 10 pm until 9 am) positive-detected blood cultures were inoculated by 9 am on a Columbia agar plate with 5 % sheep blood (COL; Becton Dickinson, Franklin Lakes, NJ, USA). Anaerobic positive blood culture bottles were inoculated on a Brucella agar plate with 5 % sheep blood (BRU; Becton Dickinson, Franklin Lakes, NJ, USA). COL and BRU agar plates were incubated at 37 °C in a 5 % supplemented CO_2 atmosphere and in an anaerobic atmosphere, respectively. Blood cultures detected positive during periods outside this time frame were not included in the study and were processed according to our standard routine identification procedures only.

The internal ethics committee of the hospital approved the anonymous use of remaining patient material.

Identification method

At 2 pm, plates were removed from the incubators for MALDI-TOF MS identification. A thin layer of growing colonies was scraped from each plate in order to fill one-third of a 10-μl plastic loop and was single-spotted on a steel target, overlaid with 1 μl of 100 % formic acid, and after drying overlaid with 1 μl matrix, a saturated solution of α-cyano-4-hydroxycinnamic acid dissolved in a basic organic solvent composed of 50 % acetonitrile and 2.5 % trifluoroacetic acid. MALDI-TOF MS measurements were realized on a MicroFlex LT platform (Bruker Daltonik, Bremen, Germany). Spectra were recorded in the positive linear method in a mass range from 2,000 to 20,000 Da, according to the manufacturer's settings. The acquired bacterial spectra with MALDI-TOF MS were analyzed in the MALDI Biotyper 3.0 software with database version 3.1.2 and bearing the spectra of 4,111 cellular organisms. Score results were interpreted according to a defined cut-off of 1.7 for acceptable identification to the species level. A score <1.7 was considered unreliable for identification. No threshold for acceptance to the genus level was defined.

The results were compared with the routine identification procedure including optochin susceptibility testing for *Streptococcus pneumoniae* suspected strains and standard MALDI-TOF MS identification from overnight culture colonies (18-h subculture) for other species isolates [18]. In this procedure, a single colony was directly plated onto a steel target and overlaid with 1 μl of matrix. According to the specifications of the manufacturer, a high log score ≥2 was required for identification to the species level and an intermediate log score lying between <2 and ≥1.7 for identification to the genus level. A low score <1.7 was considered unreliable for identification.

All result discrepancies were resolved by 16S rRNA gene sequencing according to a previously published method [19].

Bacteremic episodes

Review of the patients' medical records allowed the classification of all included positive blood culture episodes into true bloodstream infections (bacteremia/septicemia) or contaminations according to the Centers for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) surveillance definitions of specific infection types [20]. Positive blood culture bottles originating from the same patient were considered to belong to a single bacteremic episode when the difference in the sampling dates was less than 7 days.

Results

A total of 925 blood culture bottles were collected over the 7-month study period, comprising 483 aerobic broths, 377 anaerobic broths, and 65 pediatric broths.

A single microorganism grew in 896 (96.9 %) blood culture bottles, while the 29 (3.1 %) remaining bottles yielded growth of two different microorganisms (Table 1).

These 925 positive blood culture bottles corresponded to 347 bloodstream infections and 123 contaminations, and accounted for 70 % of the total positive blood cultures, excluding weekends.

Monomicrobial positive blood cultures

Of the 896 monomicrobial positive blood cultures, species identification could be obtained in 727 cases (81.1 %), including 433/527 (82.2 %) Gram-positive isolates and 292/323 (90.4 %) Gram-negative isolates (Table 2). Among the Gram-positive bacteria, staphylococci, enterococci, and streptococci were correctly identified in 351/410 (85.6 %), 45/52 (86.5 %), and 34/52 (65.4 %) positive blood cultures, respectively. For Gram-negative bacteria, 255/275 (92.7 %) of the Enterobacteriaceae and 32/34 (94.1 %) of the non-fermenters were correctly identified. On the other hand, the identification of 5-h subcultures growing with anaerobes and yeasts led to poor results, as only 2/10 and 0/36 isolates, respectively, could be identified.

In 166 positive blood cultures, the causative organisms remained unidentified by MALDI-TOF MS due either to an insufficient score for identification proposal (98 isolates) or because no peaks were detected (68 isolates). Poor growth at the 5-h subculture accounted for insufficient scores mainly in non-identified Gram-positive isolates (coagulase-negative staphylococci, viridans group streptococci, and the group of other Gram-positive organisms), while the absence of peaks could be linked to the absence of growth of yeast and anaerobes after a 5-h subculture (data not shown).

In three cases with discordant identification results compared to the routine identification procedure, 16S rRNA PCR confirmed that we had erroneously identified one *Acinetobacter baumannii* isolate as *Acinetobacter pittii* with a log score of 1.742, while two *Streptococcus* isolates (*Streptococcus salivarius* and *Streptococcus peroris*) had been misidentified as *S. pneumoniae* with log scores of 1.903 and 1.888, respectively.

Polymicrobial positive blood cultures

Rapid MALDI-TOF MS identification of the polymicrobial positive blood cultures never allowed the concomitant identification of both isolates from the same 5-h subcultured plate (Table 3). One of the two isolates was identified to the species level in 23 of the 29 blood cultures with mixed bacterial growth. One *S. peroris* strain was erroneously identified as *S. pneumoniae* with a log score of 1.998, while the scores of the five remaining positive blood cultures were insufficient to consider the identification result.

Bloodstream infections versus contamination

Among the 453 monomicrobial blood cultures, 333 corresponded to bloodstream infections, while 120 were deemed to correspond to contaminations. Species identification could be obtained for 287/333 (86.2 %) bloodstream infections and for 82/120 (68.3 %) contaminations, encompassing, respectively, 640/766 (83.6 %) and 87/130 (66.9 %) identified positive blood culture bottles.

Among the 17 mixed bacterial growths, ten polymicrobial bloodstream infections, three polymicrobial contaminations, and four monomicrobial bloodstream infections associated with a contaminating strain were defined. In two polymicrobial episodes, both strains were identified from distinct subcultured blood bottles of the same episode. In the first episode, *Staphylococcus aureus* was identified from three bottles and *Staphylococcus*

Table 1 Performance of the rapid (5-h) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification process among monomicrobial and polymicrobial bacteremic episodes

	No. of episodes	No. (%) of identified episodes with rapid MALDI-TOF MS	No. of positive blood culture isolates	No. (%) of identified isolates with rapid MALDI-TOF MS
Monomicrobial, total	453	369 (81.5)	896	727 (81.1)
Bacteremia	333	287 (86.2)	766	640 (83.6)
Contamination	120	82 (68.3)	130	87 (66.9)
Polymicrobial, total	17	0	29	0
Bacteremia	10	0	22	0
Bacteremia + contamination	4	0	4	0
Contamination	3	0	3	0
Total	470	369 (79)	925	727 (78.6)

Table 2 Identification results of monomicrobial positive blood cultures with the rapid MALDI-TOF MS process

Final identification of monomicrobial positive blood cultures with Gram-positive bacteria	*n*	Correct species identification		Final identification of monomicrobial positive blood cultures with Gram-negative bacteria, anaerobes, or yeast	*n*	Correct species identification	
		n	%			*n*	%
Gram-positive bacteria	527	433	82.2	Gram-negative bacteria	323	292	90.4
Staphylococci	410	351	85.6	Enterobacteriaceae	275	255	92.7
Staphylococcus aureus	179	171	95.5	*Citrobacter braakii*	1	0	0.0
Staphylococcus capitis	21	16	76.2	*Enterobacter aerogenes*	10	10	100.0
Staphylococcus cohnii	3	0	0.0	*Enterobacter cloacae*	17	15	88.2
Staphylococcus epidermidis	161	132	82.0	*Escherichia coli*	166	156	94.0
Staphylococcus haemolyticus	11	8	72.7	*Hafnia alvei*	7	7	100.0
Staphylococcus hominis	28	21	75.0	*Klebsiella oxytoca*	16	16	100.0
Staphylococcus lugdunensis	1	0	0.0	*Klebsiella pneumoniae*	45	39	86.7
Staphylococcus pettenkoferi	4	2	50.0	*Morganella morganii*	1	1	100.0
Staphylococcus sciuri	1	1	100.0	*Proteus mirabilis*	5	5	100.0
Staphylococcus warneri	1	0	0.0	*Salmonella* sp.	2	2	100.0
Enterococci	52	45	86.5	*Serratia marcescens*	3	3	100.0
Enterococcus avium	1	1	100.0	*Serratia rubidaea*	2	1	50.0
Enterococcus faecalis	21	17	81.0	Non-fermenters	34	32	94.1
Enterococcus faecium	30	27	90.0	*Acinetobacter baumannii*	5	4	80.0
Streptococci	52	34	65.4	*Acinetobacter pittii* (*Acinetobacter* genomospecies 3)	3	3	100.0
Pyogenic group	10	8	80.0	*Acinetobacter lwoffii*	2	2	100.0
Streptococcus pyogenes	4	2	50.0	*Pseudomonas aeruginosa*	23	22	95.7
Streptococcus agalactiae	3	3	100.0	*Stenotrophomonas maltophilia*	1	1	100.0
Streptococcus dysgalactiae	3	3	100.0	Other Gram-negative organisms	14	5	35.7
Viridans group	41	26	63.4	*Capnocytophaga sputigena*	1	0	0.0
S. anginosus group	3	1	33.3	*Haemophilus influenzae*	4	1	25.0
S. bovis group	6	2	33.3	*Moraxella catarrhalis*	1	1	100.0
S. mitis group	23	20	87.0	*Moraxella lacunata*	3	1	33.3
S. salivarius group	2	0	0.0	*Moraxella osloensis*	2	0	0.0
S. sanguinis group	7	3	42.9	*Neisseria meningitidis*	3	2	66.7
Other streptococci	1	0	0.0	Anaerobes	10	2	20.0
Granulicatella adiacens	1	0	0.0	*Actinomyces* sp.	2	1	50.0
Other Gram-positive organisms	13	3	23.1	*Bacteroides fragilis*	2	0	0.0
Aerococcus urinae	1	0	0.0	*Clostridium perfringens*	1	1	100.0
Bacillus cereus	1	1	100.0	*Leptotrichia* sp.	1	0	0.0
Corynebacterium aurimucosum	1	0	0.0	*Propionibacterium acnes*	4	0	0.0
Corynebacterium durum	1	0	0.0	Yeasts	36	0	0.0
Corynebacterium jeikeium	1	0	0.0	*Candida albicans*	17	0	0.0
Gordonia sputi	3	0	0.0	*Candida dubliniensis*	2	0	0.0
Micrococcus luteus	3	1	33.3	*Candida glabrata*	1	0	0.0
Rothia aeria	1	0	0.0	*Candida tropicalis*	10	0	0.0
Rothia mucilaginosa	1	1	100.0	*Fusarium* spp.	5	0	0.0
				Trichosporon inkin	1	0	0.0

epidermidis from a fourth bottle. Similarly, in the second episode, *Staphylococcus aureus* was identified from the first bottle and *Proteus mirabilis* from the second bottle. In 11 of the 17 mixed bacterial episodes, rapid MALDI-TOF MS identified only one of the two organisms. Nevertheless, the concomitant presence of two different isolates could be anticipated by Gram staining at the time of blood culture positivity in 6 out of 17 episodes.

Table 3 Rapid (5-h) MALDI-TOF MS identification results of the 17 polymicrobial bacteremic episodes. Clinically relevant pathogens are reported in **bold**

Episode	Gram staining lecture	Rapid MALDI-TOF MS identification results		Final identification results
		Identified/ total BCB	Identified strain(s)	
Polymicrobial bloodstream infection				
1	GPC	3/6 and 1/6	*Staphylococcus aureus*/ *Staphylococcus epidermidis*	***Staphylococcus aureus*** + ***Staphylococcus epidermidis***
2	GPC in chains + GNB	2/2	*Escherichia coli*	***Enterococcus gallinarum*** + ***Escherichia coli***
3	GPC in chains + GNB	2/2	*Escherichia coli*	***Enterococcus faecium*** + ***Escherichia coli***
4	GPC in clusters	2/2	*Enterococcus faecalis*	***Enterococcus faecalis*** + ***Enterococcus faecium***
5	GPC in clusters	2/2	*Enterococcus faecalis*	***Enterococcus faecalis*** + ***Acinetobacter baumannii***
6	GPC in clusters	2/2	*Staphylococcus aureus*	***Staphylococcus aureus*** + ***Staphylococcus epidermidis***
7	GPC in clusters + GNB	2/2	*Escherichia coli*	***Streptococcus pyogenes*** + ***Escherichia coli***
8	GPC in clusters + GNB	1/2 and 1/2	*Staphylococcus aureus*/ *Proteus mirabilis*	***Staphylococcus aureus*** + ***Proteus mirabilis***
9	GNB	1/1	*Escherichia coli*	***Escherichia coli*** + ***Proteus mirabilis***
10	GNB	1/1	*Klebsiella oxytoca*	***Escherichia coli*** + ***Klebsiella oxytoca***
Monomicrobial bloodstream infection + contamination				
11	GPC in chains	0/1	–	*Staphylococcus epidermidis* + ***Enterococcus faecalis***
12	GPC in chains	1/1	*Streptococcus agalactiae*	*Staphylococcus epidermidis* + ***Streptococcus agalactiae***
13	GPC in chains + clusters	1/1	*Streptococcus oralis*	***Staphylococcus aureus*** + *Streptococcus oralis*
14	GPC in clusters	0/1	–	*Staphylococcus epidermidis* + ***Escherichia coli***
Polymicrobial contamination				
15	GPC	1/1	*Staphylococcus hominis*	*Staphylococcus hominis* + *Staphylococcus capitis*
16	GPC in chains + clusters	0/1	*Streptococcus pneumoniae*	*Streptococcus peroris* + *Staphylococcus capitis*
17	GPC in clusters	0/1	–	*Staphylococcus epidermidis* + *Aerococcus urinae*

BCB blood culture bottles; *GNB* Gram-negative bacilli; *GPC* Gram-positive cocci

Discussion

We evaluated here a practical approach for the rapid identification of microorganisms growing from positive blood cultures in daily routine clinical practice. Numerous studies have already assessed the performance of MALDI-TOF MS procedures for rapid microorganism identification when directly applied on culture-positive blood specimens. Accurate species identification rates were found to vary between 50.5 % and 91 %, depending both on the distribution of microbial isolates and on the applied pretreatment/extraction methods, as well as on the definitions of the cut-off threshold log scores [7, 9–15]. However, direct bacterial identification by MALDI-TOF MS from positive blood cultures is time- and labor-intensive, since it requires at least 30 min hands-on time for the washing, centrifugation, and extraction steps that are necessary to discard blood cells and reveal the bacterial proteins. Despite the possibility of obtaining, in theory, a result within 60 min from the time a positive blood culture is detected, the proposed workflow is difficult to integrate in the routine workflow of a clinical microbiology laboratory. Hence, direct MALDI-TOF MS identification of positive blood cultures is most usually realized in batches, for instance, every 2 h, as suggested by Loonen et al., or twice a day, according to Martiny et al., thereby extending the time to obtaining identification results [9, 10].

In this study, we investigated an identification procedure not requiring any additional time- or labor-consuming sample preparation steps and leading to identification results available to the clinician within the same day as blood culture positivity. Our MALDI-TOF MS processing algorithm after a 5-h subculture from positive blood bottles with formic acid overlay as the only preparation step could be considered as an intermediate method between the direct MALDI-TOF MS identification process and the "next-day" MALDI-TOF MS identification from an 18-h subculture. McElvania TeKippe et al. previously evaluated the formic acid overlay process for the MALDI-TOF MS identification of Gram-positive cultured organisms and showed a significant improvement of genus- and species-level identification (by 20 %) and higher scores compared to the direct smear deposit [21]. Ford and Burnham similarly demonstrated the added value of the formic acid overlay versus the direct smear method for the identification of Gram-negative bacterial colonies by the reduction of unidentified organisms [22].

In our evaluation, the processing time of subcultures was set at 5 h after preliminary MALDI-TOF MS identification. Experiences following 3 and 4 h of incubation were found to be associated with very poor identification results for Gram-positive isolates and only moderate results for Gram-negative strains (data not shown). Idelevich et al. similarly evaluated rapid MALDI-TOF MS identification of microorganisms from positive blood cultures subsequent to a 1.5-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 12-, and 24-h incubation on solid medium [23]. The mean incubation time needed to achieve species-level identification was 5.9 and 2 h for Gram-positive aerobic cocci (n=86) and Gram-negative aerobic rods (n=42), respectively.

For monomicrobial positive blood cultures, species identification results could be achieved for 81.1 % of the isolates, which can be considered as a very satisfactory result when compared to other recent studies using direct MALDI-TOF MS, in which correct identification ranged between 64.8 and 81.8 % [11, 14, 15]. In line with these authors, we also noticed a higher identification percentage for Gram-negative organisms (90.4 %) compared to Gram-positive organisms (82.2 %). As defined in the Materials and methods section, identification results were accepted to the species according to a cut-off score ≥1.7. Using a cut-off score ≥2 for species identification allowed correct identification results for 69.6 % of the monomicrobial positive blood cultures and identified one of the two strains in 21 of the 29 polymicrobial positive blood cultures. No isolates were erroneously identified. A less stringent cut-off score ≥1.5 for species identification allowed results for 84 % of the monomicrobial positive blood cultures and identified one of the two strains in 24 of the 29 polymicrobial positive blood cultures. Three isolates were erroneously identified, as observed with the cut-off score at 1.7. The final choice to set the cut-off at 1.7 in our study was taken in accordance with the abundant publications using this scoring system when direct MALDI-TOF MS identification is applied [9, 17, 24].

One drawback of our rapid MALDI-TOF MS process was that it failed to yield correct identification results for yeast and anaerobes most probably related to the insufficient growth of these microorganisms on the agar plates after a 5-h subculture. Pondering the high rates of morbidity and mortality as well as the growing incidence of candidemia and anaerobic septicemia, a prompt identification result is essential [25, 26]. Hence, to overcome this flaw in our process, direct analysis from positive blood culture samples by MALDI-TOF MS should be considered systematically when Gram staining suggests the presence of yeast or anaerobes. This procedure preceded by defined blood lysing protocols using sodium dodecyl sulfate detergent or Tween 80 and formic acid extraction respectively enabled Pulcrano et al. [27] to identify 19/21 *Candida* non-*albicans* bloodstream infections and Leli et al. to identify 7/7 anaerobic septicemia [15. The erroneous identification of two streptococci (*S. salivarius* and *S. peroris*) as *S. pneumoniae* in our study confirmed the inability of MALDI-TOF MS to distinguish oral streptococci strains from *S. pneumoniae* [18, 28, 29]. On the basis of these observations, we decided not to consider any *S. pneumoniae* result through our rapid MALDI-TOF MS identification process. The third erroneously identified strain was an *A. baumannii* isolate that was misidentified to the species level as *Acinetobacter pittii* (formerly *Acinetobacter* genomospecies 3). In the MALDI Biotyper 3.0 software, *Acinetobacter* species identification results are accompanied with a comment informing about the close relatedness of several species and the difficulty in differentiating them.

The main weakness of our MALDI-TOF MS protocol was its inability to identify all organisms in the setting of polymicrobial bloodstream infections. Various studies also underlined the lack of ability of MALDI-TOF MS to detect all microorganisms in mixed cultures through direct identification, as none or, at best, one single isolate could be identified [10–12, 15]. Ferroni et al. managed the identification of blood cultures containing mixed bacteria through the use of Gram-specific databases selected according to the obtained Gram result [7]. In our setting, Gram staining of all positive blood culture bottles and rapid MALDI-TOF MS identification of all subcultured isolates included in the polymicrobial episodes were essential elements that partially overcame this limitation of our algorithm. Regarding the possibility of detecting polymicrobial bacteremia, visualization of all plated blood subcultures was systematically repeated the day following positivity considering that the presence of more than one organism could go undetected on young subcultures.

The ultimate objective of this study was to speed up the identification process for improving the management of the patient and to assist the clinician in deciding whether the growing microorganisms were to be considered as clinically relevant and associated with a bloodstream infection or, rather, whether they should be considered as contaminants. Overall, 86.2 % of the monomicrobial bloodstream infections could be identified, thereby potentially allowing an earlier diagnosis and adaptation of therapy to the documented pathogens. In parallel, 68.3 % of all organisms regarded as clinically non-significant contaminants could be identified and reported on the same day of blood culture positivity, possibly leading to restriction and/or earlier stop of antimicrobial therapy. Martiny et al. measured the clinical impact of rapid microbial identification (direct MALDI-TOF MS preceded by an in-house purification protocol) on the management of septic patients. An accelerated modification of the treatment regimen was observed in 13.4 % and 2.5 % of the adult and pediatric patients, respectively. In other cases, the tool was helpful to rapidly confirm suspected cases of contamination, thereby avoiding the administration of unnecessary antibiotics [16]. Vlek et al., likewise, observed an 11.3 % increase in the proportion of patients receiving appropriate antibiotic

treatment 24 h after blood culture positivity with direct MALDI-TOF MS performed twice a day [17].

These results emphasize the benefit of the rapid identification of positive blood cultures compared to MALDI-TOF MS analysis on 18-h incubated colonies the day after blood culture positivity detection.

Considering the satisfactory identification results and the potentially favorable clinical impact on patient management, a routine-applicable positive blood culture work scheme integrating MALDI-TOF MS identification on young positive blood subcultures was implemented as presented in Fig. 1. Three time frames were defined according to the time of day during which growth-positive blood cultures were detected by the automated culture system. MALDI-TOF MS analysis on short subcultures was applied at 5 pm for all bottles detected positive between 0 am and 12 am, thereby allowing the report of the results to clinicians at 5.30 pm. A direct MALDI-TOF MS identification (Sepsityper, Bruker Daltonik, Bremen, Germany) was executed for positive-detected blood cultures between 12 am and 5 pm. This commercial method had been previously validated in our university hospital, allowing 65.3 % correct identifications (data not shown). Positive blood culture bottles detected between 5 pm and 0 am were subcultured but only identified on the following day according to the standard MALDI-TOF MS identification process. During weekends, the short subculture MALDI-TOF MS identification was applied once daily at 2 pm, allowing results reporting of all blood cultures detected positive until 9 am. Gram staining was systematically performed on all positive-detected blood bottles and immediately communicated to the clinicians between 9 am and 00 am every day of the week.

We believe that a major strength of this algorithm is the gain in hands-on time and cost if compared with systematic direct MALDI-TOF MS analysis while preserving the gain in time in positive blood culture identification result reporting. On weekdays, the results were systematically communicated by phone at 5.30 pm to the infectious diseases physicians team, potentially allowing faster antimicrobial treatment modifications, as previously demonstrated by several authors [16, 17].

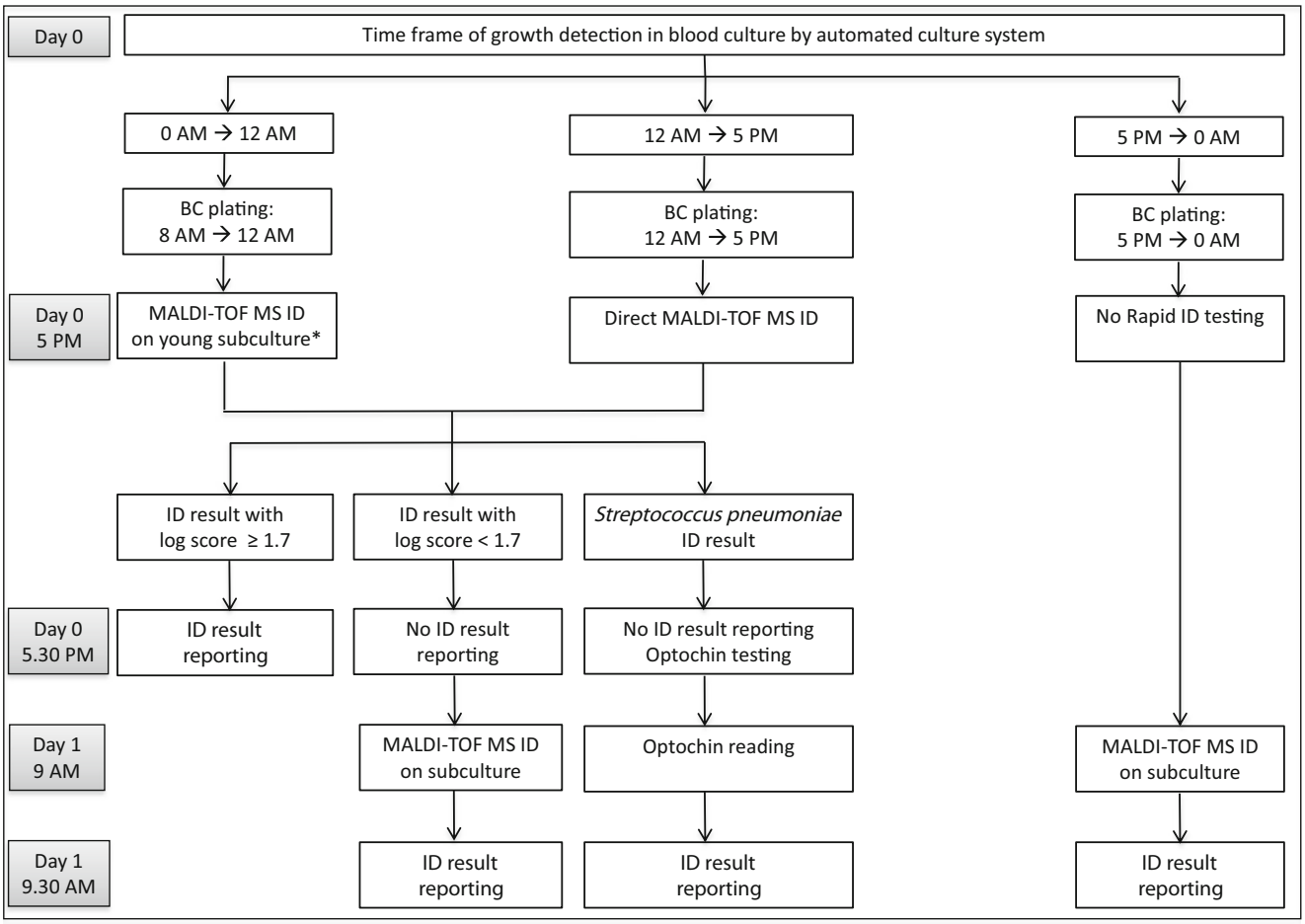

Fig. 1 Modified weekday routine workflow scheme for the identification of blood cultures in accordance with the time of positivity detection by theautomated incubation system. *Direct MALDI-TOF MS ID when Gram staining suggestive of yeast or anaerobes. *BC* blood culture; *ID* identification; *MALDI-TOF MS* matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

The large amount of tested isolates, thereby representative of the routine positive blood culture microorganism proportions in a tertiary hospital, enabled us to validate the applied process. All during the study period, testing was carried out by various technologists and medical junior residents, thereby highlighting the robustness and reproducibility of the method in clinical routine practice.

In conclusion, the integration of MALDI-TOF MS identification on 5-h subcultured colonies in the laboratory workflow represents an excellent compromise between the direct blood culture process associated with labor-intensive steps and the direct smear method of 18-h subcultured colonies, as it leads to the reporting of correct identification results on the day of positivity in more than 80 % of the monomicrobial bacteremic episodes. An ongoing challenge is the development of rapid tests for the detection of clinically important resistance mechanisms, since we should keep in mind that the identification results alone only give partial microbiological information to the clinicians. Hence, the impact on the patient's clinical management of the rapid positive blood culture identification result "alone" may also be very dependent on the local epidemiology of bacterial resistance. Indeed, the increasing trends of resistance of Gram-negative bacteria to third-generation cephalosporins and to carbapenems, as well as the high rates of methicillin-resistant *Staphylococcus aureus* across Europe, remind us that clinicians can no longer simply rely on the wild susceptibility profile of the identified bacteria for therapeutic decision-making [30]. A study is actually ongoing in our hospital to assess whether the combination of rapid MALDI-TOF MS identification associated with rapid detection of resistance mechanisms to selected antimicrobial agents may favorably impact on antimicrobial therapy among septic patients with positive blood cultures.

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Conflict of interest The authors declare that they have no conflict of interest.

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