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ORIGINAL ARTICLE

Rapid identification by MALDI-TOF/MS and antimicrobial disk diffusion susceptibility testing for positive blood cultures after a short incubation on the WASPLab

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Abstract

The objectives of this study were to define the shortest incubation times on the WASPLab for reliable MALDI-TOF/MS-based species identification and for the preparation of a 0.5 McFarland suspension for antimicrobial disk diffusion susceptibility testing using short subcultures growing on solid culture media inoculated by positive blood cultures spiked with a wide range of pathogens associated with bloodstream infections. The 520 clinical strains (20 × 26 different species) included in this study were obtained from a collection of non-consecutive and non-duplicate pathogens identified at Geneva University Hospitals. After 4 h of incubation on the WASPLab, microorganisms' growth allowed accurate identification of 73% (380/520) (95% CI, 69.1–76.7%) of the strains included in this study. The identification rate increased to 85% (440/520) (95% CI, 81.3–87.5%) after 6-h incubation. When excluding *Corynebacterium* and *Candida* spp., the microbial growth was sufficient to permit accurate identification of all tested species (100%, 460/460) (95% CI, 99.2–100%) after 8-h incubation. With the exception of *Burkholderia cepacia* and *Haemophilus influenzae*, AST by disk diffusion could be performed for Enterobacterales and non-fermenting Gram-negative bacilli after only 4 h of growth in the WASPLab. The preparation of a 0.5 McFarland suspension for Gram-positive bacteria required incubation times ranging between 3 and 8 h according to the bacterial species. Only *Corynebacterium* spp. required incubation times as long as 16 h. The WASPLab enables rapid pathogen identification as well as swift comprehensive AST from positive blood cultures that can be implemented without additional costs nor hands-on time by defining optimal time points for image acquisition.

Keywords Copan WASPLab · MALDI-TOF/MS · Short subcultures · AST by disk diffusion · Automation · Accuracy · Turn-around time

Introduction

One of the most important tasks performed by clinical microbiology laboratories is the accurate identification and antimicrobial susceptibility testing (AST) of pathogens growing in blood cultures and responsible for systemic infection. In patients with severe sepsis and septic shock, early administration of appropriate antibiotics contributes to optimal patient management by reducing mortality, shortening the length of hospital stay, and preventing the development of antibiotic resistance [1–3]. Delays in introducing adequate antimicrobial therapy were correlated with a 7.6% decrease in survival for a septic patients [4]. While clinical symptoms, epidemiological information, and Gram-staining results can guide empiric antimicrobial therapy, the final antimicrobial therapy could hinge on the pathogen identification and AST. This is particularly true when it comes to those organisms that may not be covered by typical empiric regimens (e.g., *Stenotrophomonas*). Furthermore, every empiric broad-spectrum antimicrobial prescription can conceivably contribute to the selection of multidrug-resistant organisms, especially that multidrug-resistant Gram-negative bacteria is the main

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cause of empiric antimicrobial therapy failure in bloodstream infection and sepsis [5, 6]. Swift de-escalation of broad-spectrum antibiotics based on earlier diagnosis must therefore help improve patient outcome and infection control. In Geneva University Hospitals, all patients with positive blood cultures are closely monitored by certified infectious diseases specialists in assistance for the attending doctors in order to tailor antimicrobial choice, dosing regimen, and duration of therapy.

Blood cultures (BCs) remain the gold standard to identify pathogens associated with bloodstream infection and to perform antimicrobial susceptibility testing (AST). BCs rely on a series of conventional methods, including Gram-staining, subculture, and different techniques for strain identification and AST. The whole process can last between 24 and 72 h (in the case of anaerobic bacteria). According to published studies, BCs allow the identification of the pathogens in one third of the septic cases, which makes the processing of BCs, microorganism's identification, and AST at the top list of laboratory priorities [7, 8]. Improving the management of BCs begins with the optimization of the pre-analytic steps, like the time required for bottles transport and for the insertion of such bottles into the automated blood culture monitoring systems. Although several strategies have been employed to expedite the identification process, no satisfactory method is available for routine clinical use. Thus, there is an ongoing quest for a simple, rapid, broad-spectrum, and cost-effective system for the direct and rapid identification of BC pathogens.

The purpose of this study was to define the shortest incubation times on the WASPLab for reliable MALDI-TOF/MS-based species identification and for swift AST by disk diffusion starting from short subcultures growing on solid culture media inoculated from positive blood cultures.

Material and methods

Bacterial strains

The 520 clinical strains included in this study were taken from a collection of non-consecutive and non-duplicate pathogens commonly associated with bloodstream infections and diagnosed in the bacteriology laboratory of Geneva University Hospitals. All isolates were stored at − 80 °C in skim milk with 15% glycerol. The identification of the strains was performed by matrix-assisted desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS Compass, Bruker Daltonics, Bremen, Germany) according to the manufacturer's instructions. The matrices used in this study were obtained from non-consecutive blood culture samples referred to the clinical bacteriology laboratory at Geneva University Hospitals between August and November 2019 and ultimately diagnosed as negative BC samples.

Preparation of the spiked samples

This evaluation was performed by spiking common pathogens (20 non-duplicate clinical isolates for each of the 26 species; Table 1) associated with previous bloodstream infections into negative BC bottles in order to mimic positive blood cultures. The inoculum suspensions were prepared by selecting several colonies from overnight growth on corresponding agar plates with a cotton swab and suspending the colonies in sterile saline to the density of a 0.5 McFarland standard, corresponding to approximately 10^8 CFU/ml. About 200 μl of different inoculum suspensions were injected into negative blood culture bottles using BD BACTEC™ Plus Aerobic medium or BD BACTEC™ Plus Anaerobic medium. Inoculated bottles were immediately placed in the BD BACTEC™ FX. Individual blood culture bottles were removed from the automated blood culture system when growth was detected (defined as time to positivity, ranging between 6 and > 24 h), and the sample was proceeded on the WASPLab.

Sample processing

About 6 ml of blood culture fluid were aspirated from each positive bottle with sterile needle/syringe and transferred into a sterile tube in order to be inoculated by the WASP. The blood culture fluid (1 x 30 μl loop/spreader) was spread over the entire surface of Columbia agar with 5% sheep blood (Becton Dickinson), Columbia CNA agar with 5% sheep blood (Becton Dickinson), chocolate agar (bioMérieux), and MacConkey agar (Becton Dickinson), according to the streaking pattern defined by COPAN, and the culture media plates were transferred into the WASPLab incubators. We determined the shortest incubation times on the WASPLab (Copan WASP srl, Brescia Italy) required for MALDI-TOF/MS-based species identification, and for standard AST by disk diffusion, by performing time-series image acquisition on the WASPLab at sequential time points (2, 3, 4, 6, 8, 12, 16, 20, 24, 30, 40, 50, 60, and 72 h). For each incubation time point, several high-resolution digital images were acquired under different light and exposure conditions according to the manufacturer's instructions. Identification by MALDI-TOF/MS was performed at the first incubation time point which showed sufficient microbial growth. If the identification was considered as reliable, no further identification was attempted during the following time points. However, if the identification was not reliable, another identification was performed at the next time point until the MALDI-TOF/MS provided a reliable identification, according to the manufacturer's instructions. The same approach was applied for the AST. A 0.5 McFarland suspension for standard AST by

Table 1 Minimal incubation times required for MALDI-TOF /MS-based species identification and AST by disk diffusion from short subcultures growing on solid media incubated on the Copan WASPLab

Microorganisms		Number of non-duplicate strains analyzed	Incubation time required for MALDITOF/MS-based species identification from short subcultures growing on solid media (hours)	Incubation time required for 0.5 McFarland suspension from short subcultures growing on solid media for AST by disk diffusion (hours)
Enterobacteriaceae	*Escherichia coli*	20	2	2
	Klebsiella pneumoniae	20	2	2
	Proteus mirabilis	20	2	2
	Salmonella	20	3	3
Non-fermenting Gram-negative bacilli	*Pseudomonas aeruginosa*	20	3	3
	Stenotrophomonas maltophilia	20	4	4
	Acinetobacter spp.	20	4	4
	Burkholderia cepacia	20	8	8
Gram-negative coccobacilli and othe Gram-negative bacilli	*Haemophilus influenzae*	20	6	6
	Pasteurella spp.	20	4	4
	Aeromonas spp.	20	3	3
Staphylococcus	*Staphylococcus aureus*	20	4	4
	Staphylococcus epidermidis	20	4	4
Streptococcus	*Streptococcus pneumoniae*	20	3	4
	Streptococcus agalactiae	20	3	4
	Streptococcus mitis	20	4	6
	Streptococcus pyogenes	20	3	4
Nutritionally deficient bacteria	*Abiotrophia*	20	6	8
	Granulicatella adiacens	20	6	8
Enterococcus	*Enterococcus faecalis*	20	3	3
	Enterococcus faecium	20	3	3
Gram-positive aerobic bacilli	*Listeria monocytogenes*	20	4	6
	Bacillus spp.	20	4	4
	Corynebacterium spp.	20	16	16
Yeast	*Candida glabrata*	20	6 (sufficent yeast biomass but no reliable identification)	
	Candida albicans	20		

disk diffusion was carried out at the first time point when the growth was considered as sufficient.

Results

MALDI-TOF/MS-based species identification

Table 1 depicts the minimal incubation times required to identify 26 different pathogens commonly associated with bloodstream infections using MALDI-TOF/MS. About 4 h was sufficient to accurately identify the most frequent pathogens associated with bloodstream infections, i.e., *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. We can illustrate the importance of this shortened incubation times by taking the example of our laboratory data. Among the 76'547 blood culture bottles referred to the bacteriology laboratory of Geneva University Hospitals during the year 2018, 6% were positive. Among them, *E. coli*, *K. pneumoniae*, *S. aureus*, and *S. epidermidis* constituted 49% of all microorganisms isolated from blood cultures. After 4h incubation on the WASPLab, microorganisms' growth allowed accurate identification of 73% (380/520) (95% CI, 69.1–76.7%) of the strains included in this study. The identification increased to 85% (440/520) (95% CI, 81.3–87.5%) after 6-h incubation. When excluding *Corynebacterium* and *Candida* spp., the microbial growth was sufficient to permit accurate identification of all tested species (100%, 460/460) (95% CI, 99.2–100%) after 8 h (Table 1 and Figs. 1, 2, 3, 4).

Importantly, the identification by MALDI-TOF/MS of *Streptococcus pneumoniae* and *Streptococcus mitis*

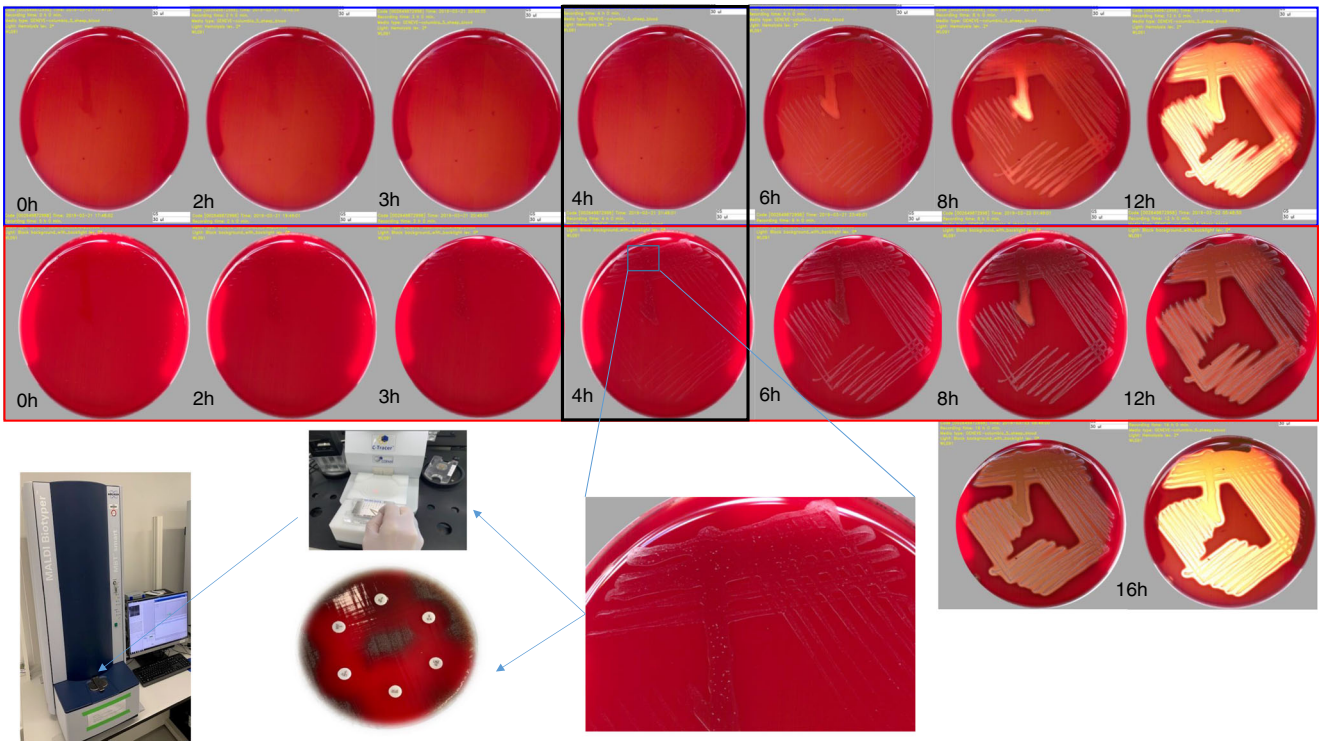

Fig. 1 High-resolution digital images taken at sequential time points on blood agar media inoculated with blood culture positive for *Streptococcus pyogenes*

should be confirmed by another method, as recommended by Bruker. This intermediate information nonetheless makes it easier to exclude the presence of an *Enterococcus*. The presence of *Candida* revealed also

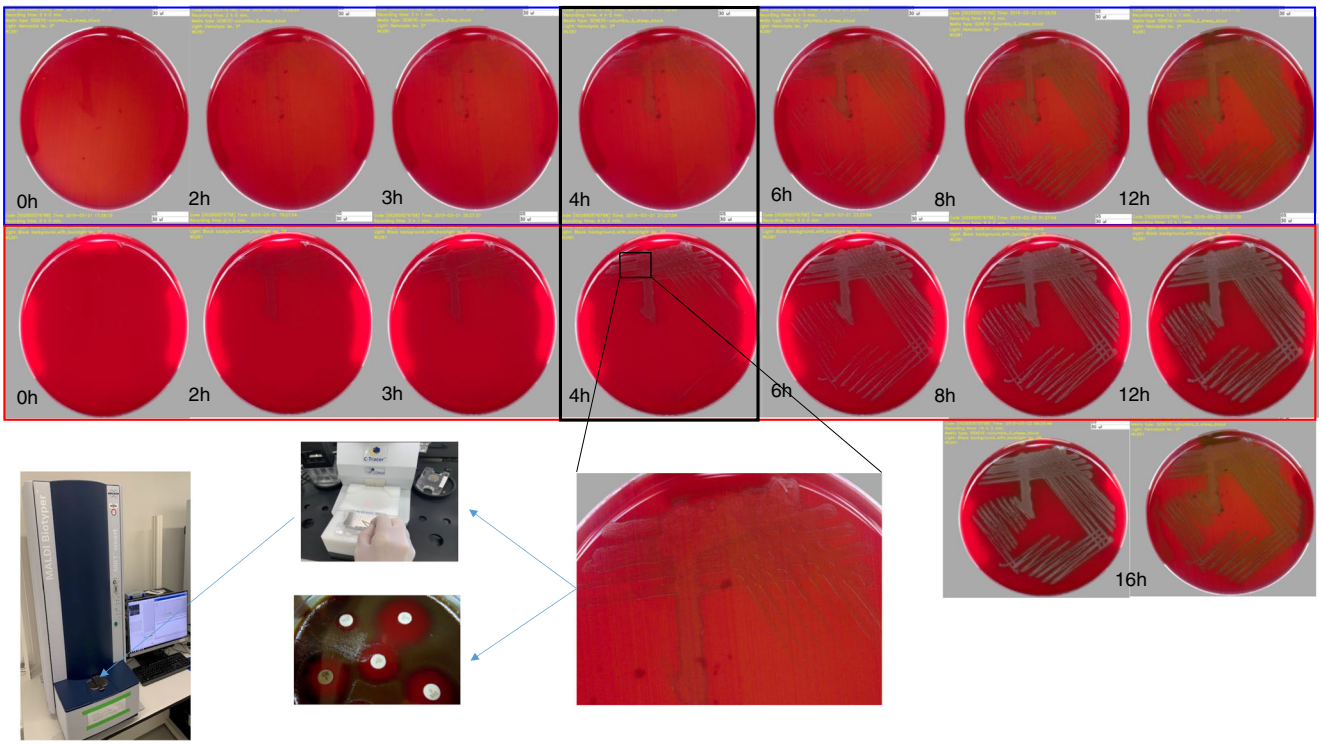

Fig. 2 High-resolution digital images taken at sequential time points on blood agar media inoculated with blood culture positive for *Streptococcus pneumoniae*

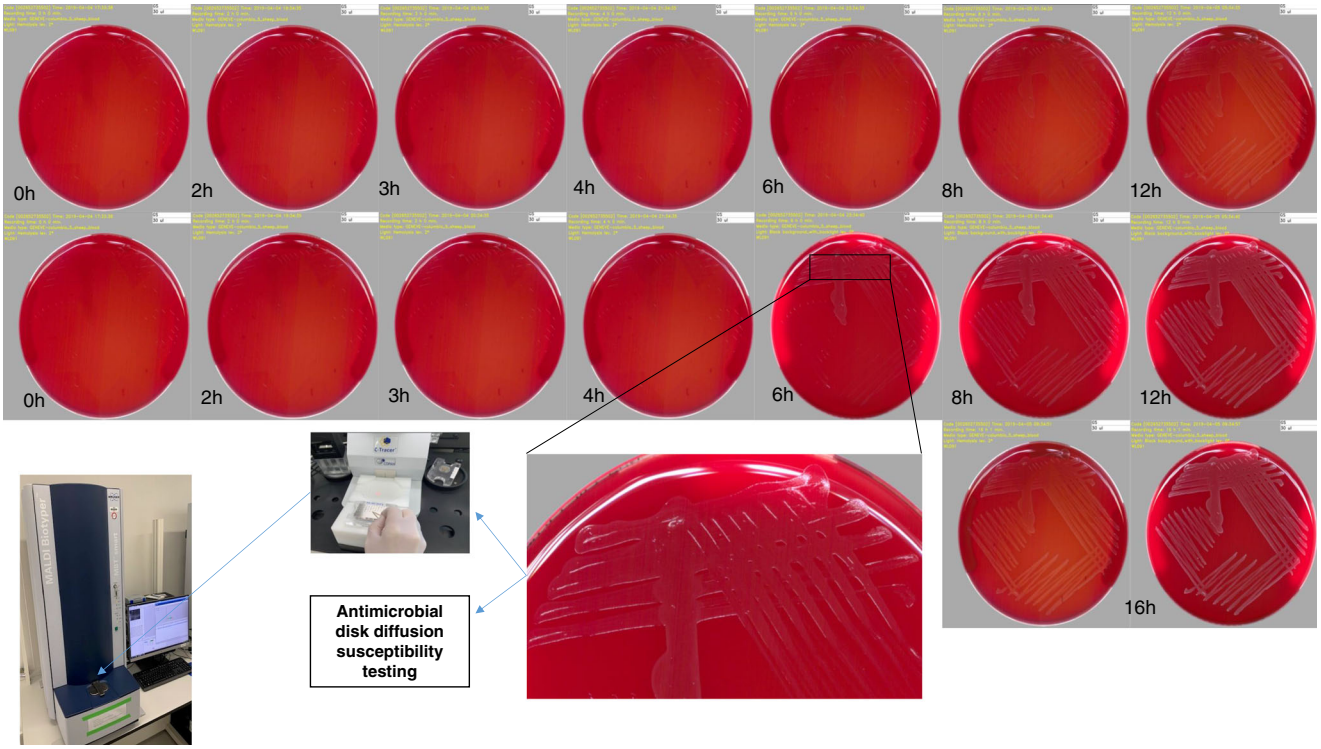

Fig. 3 High-resolution digital images taken at sequential time points on blood agar media inoculated with blood culture positive for *Listeria monocytogenes*

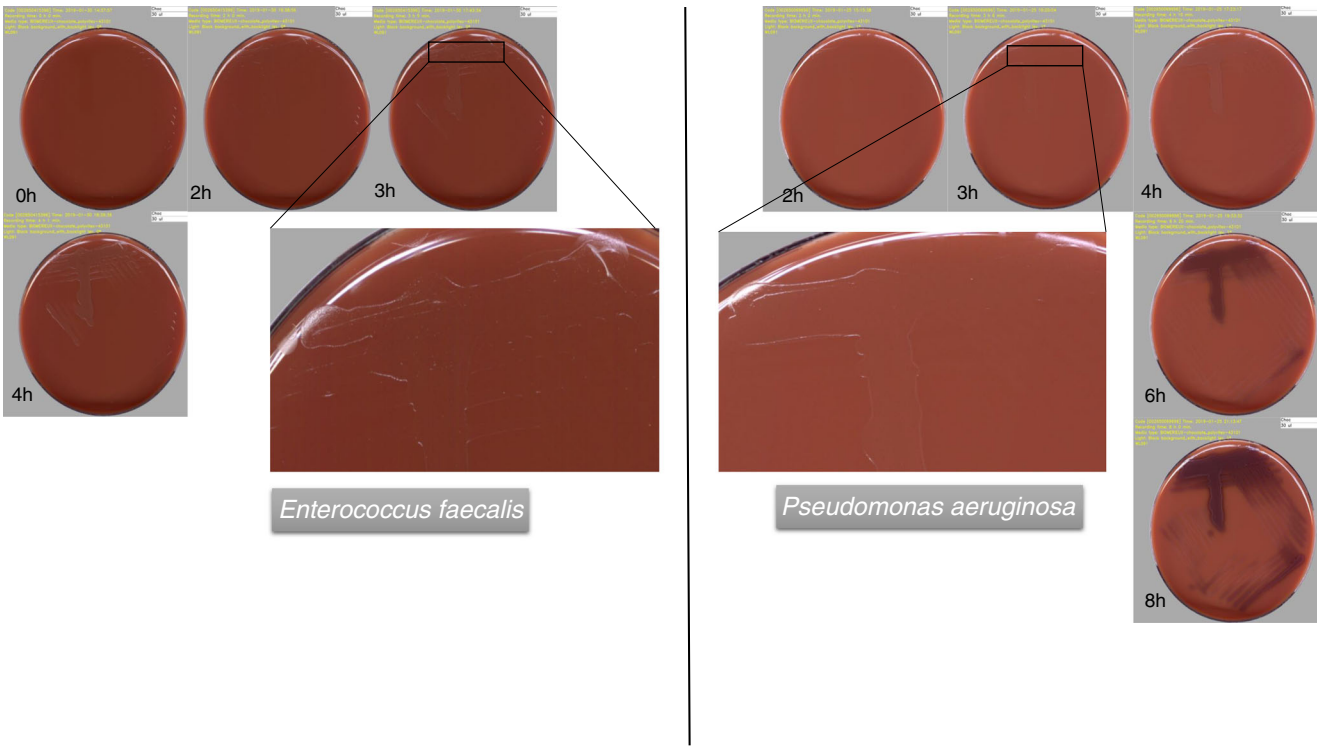

Fig. 4 High-resolution digital images taken at sequential time points on chocolat media inoculated with blood cuture positive for *Enterococcus faecalis* (left) or *Pseudomonas aeruginosa* (right)

challenging as its identification from the short subcultures was often not reliable. However, it should be noted that the issue with the identification of *Candida* will in fact be compensated by notifying rapidly the attending physicians about Gram-stain findings when blood cultures turn positive.

Antimicrobial susceptibility testing by disk diffusion

The objective of this section was to assess the shortest incubation times required to have sufficient biomass to prepare a 0.5 McFarland suspension for antimicrobial disk diffusion susceptibility testing. With the exception of *Burkholderia cepacia* and *Haemophilus influenzae*, AST by disk diffusion could be performed for Enterobacterales and non-fermenting Gram-negative bacilli after only 4 h of growth in the WASPLab. Preparing a 0.5 McFarland suspension for Gram-positive bacteria required incubation times ranging between 3 and 8 h according to the bacterial species. Only *Corynebacterium* spp. required incubation times as long as 16 h. Importantly, besides *Streptococcus*, nutritionally deficient bacteria (e.g., *Granulicatella adiacens*), and *Listeria monocytogenes*, we did not observe relevant differences between the incubation times required for the MALDI-TOF/MS-based identification and for the preparation of the inoculum from the short subcultures (Table 1 and Figs. 1, 2, 3, 4). This observation is crucial for building the laboratory workflow as it allows defining a unique incubation time for the identification and the inoculum preparation for AST.

Discussion

During the last decade, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF/MS) has revolutionized the identification of pathogens in clinical microbiology laboratories [9]. Various preparation methods have been reported for the identification of microorganisms directly from positive blood culture fluids using MALDI-TOF/MS. In essence, the principle is to lyse the human blood cells followed by a filtration or a centrifugation procedure to separate and enrich the microorganisms. MALDI-TOF/MS is then performed on such concentrate [10–12]. Even though its undeniable ability in enhancing a time to results, which is potentially linked to the improvement in patient outcomes, these methods have several limitations including reagent costs, need of an important volume of blood culture broth, high hands-on time dedicated and lack of reproducible accuracy for Gram-positive bacterial identification, as well as lack of viable microorganisms hampering the direct AST from such concentrates [13–15]. Moreover, these methods can be difficult to embed into a common routine laboratory workflow, thus either reducing their medical value or contributing to suboptimal and costly laboratory workflows. An alternative approach consists of performing the identification by MALDI-TOF/MS and AST on short subcultures (i.e., less than 6 h). This approach seems to identify correctly more than 80% of the tested species and to provide reliable AST results [16–21]. Again, the implementation of this approach in non-automated clinical microbiology laboratories requires additional manual reading steps, all of which can increase significantly the workload and affect the laboratory workflow (e.g., dedicated technicians, hours of operation, etc.). MALDI-TOF/MS was also used to detect antibiotic resistance. Various protocols have been published to detect specific enzymatic activities on bacterial extracts [22, 23]. These methods are limited by high hands-on time, the need for accurate calibration of the mass spectrometer, the limited number of resistance mechanisms that can be assessed, as well as the impossibility to identify inducible resistance or resistance related to other mechanisms (e.g., porin, efflux pumps, penicillin-binding proteins).

Molecular approaches, such as DNA hybridization or rapid PCR-based test, have been integrated in clinical microbiology laboratories with the purpose of improving the sensitivity and specificity of the assays, of reducing the time for identification of microorganisms isolated from blood cultures, and of containing infectious disease outbreaks. In this context, numerous multiplex assays have been developed to investigate a panel of targets [24, 25]. However, these PCR approaches remain expensive and limited to known and poorly polymorphic gene resistance markers. The advent of next-generation sequencing might facilitate throughput and breadth of target determinations, but it currently remains limited by its costs and the need for a validated bioinformatics pipeline [26, 27].

Presumably, shortening the length of time before obtaining blood culture analysis results could positively improve the patient's outcome. This implies providing earlier results to the physicians to help them simplify treatment decisions, including switches from empiric to targeted regimen. This in turn implies using these established incubation times to build appropriate and specific workflows according to the laboratory hours of operation. It is important to stress that not all laboratories can operate 24/7 and have therefore to adjust their workflows during opening hours to maximize benefits from the automation. However, the real impact of these procedures on medical decisions will also strongly depend on the responsiveness of the medical teams when the results are available and properly communicated on the laboratory information system.

Conclusions

In this study we established the shortest incubation times on the WASPLab for MALDI-TOF/MS-based identification and

inoculum preparation for AST for a wide panel of commonly encountered pathogens causing systemic infections. Turnaround times can be improved without additional costs or additional hands-on time by applying the defined time points for image analysis and by building an improved laboratory workflow according to the laboratory hours of operation. An important limitation of this study relates to the fact that we analyzed spiked negative blood cultures, but this permitted to include a wide range of pathogens associated with bloodstream infections. In addition, we did not analyze polymicrobial blood cultures. Further studies focusing on large series of routinely obtained positive blood culture are now warranted.

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Compliance with ethical standards

Conflict of interest JS received restricted research grants and participated to advisory boards from BioMérieux and Debiopharm. NV received restricted research grants from Roche. This research was conducted in the absence of any commercial or financial relationships in the last 3 years that could be construed as a potential conflict of interest.

Ethical approval In accordance with the local ethical committee (Commission cantonale d'éthique de la recherche, https://www.hug-ge.ch/ethique), routine clinical laboratories of our institution may use biological sample leftovers for method development after irreversible anonymization of the data.

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