1 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** avseende ?

Välj det alternativ som är MEST korrekt:

- Genom så kallad korttids-odling krävs inte identifiering med MALDI-ToF MS.
- En av fördelarna med blododling är att det inte är en tidsbegränsande metod.
- Den vanligaste metoden för att identifiera mikroorgansimer i blod är blododling.
- Med så kallad korttids-odling så kan mikroorganismer i blod identifieras snabbare.
- Gold standard för detektion av mikroorganismer i blod är MALDI-ToF.

Totalpoäng: 1

2 Läs bifogad artikel och besvara följande fråga:

Vilket var syftet med studien?
Välj det alternativ som är MEST korrekt:

- Att etablera satellit verksamheter för blododling samt analys direkt efter blodprovtagning.
- Att studera betydelsen av transporttiden för positiva blododlingskulturer.
- Att undersöka om transporttid i rumstemperatur påverkar utfallet vid korttids-blododling och MALDI-ToF MS.
- Att kvantifiera effekten av förlängd analystid av blododlingspositiva kulturer via MALDI-ToF.
- Att utveckla en ny metod för identifiering av mikroorganismer i positiva blododlingskulturer.

Totalpoäng: 1

3 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?

Välj ett alternativ:

- Till studien användes bland annat sex vanligt förekommande gram negativa bakterier, såsom *Escherichia coli* och *Staphylococcus aureus.*
- Till de stimulerade blodkulturerna användes lika många *Escherichia coli* isolat som Staphylokock isolat.
- Stimulerade blodkulturer inkuberades i BacT/Alert Virtuo automatiserat blododlingssystem.
- Det vanligaste bakterieiolatet som användes till de stimulerade blodkulturerna var *Klebsiella pneumoniae*
- Till studien användes de mikrobiologiska patogen som är mest virulenta samt associerade med allvarliga tillstånd.

Totalpoäng: 1

4 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?
Välj ett alternativ:

- Bakteriestammarna resuspenderades i 0.9% NaCl till en turbiditet på 0.3 McFarland.
- Totalt så användes 257 simulerade blodkulturflaskor.
- Till de stimulerade blodkulturerna användes 15,000 CFU.
- För att kontrollera antalet CFU användes tre blodagarplattor per simulerad blodkultur.
- Av de simulerade blododlingskulturerna odlades 48 anaerobt och 209 aerobt.

Totalpoäng: 1

5 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?
Välj ett alternativ:

- Standardtid för korttids-blododling på agarplattor var fyra timmar.
- Efter korttids-odling så analyserades samtliga prover i duplikat med MALDI-ToF MS.
- För att efterlikna tid för transport så inkuberades de simulerade blododlingsflaskorna i 48 timmar i rumstemperatur innan odling.
- MALDI-ToF MS scores ≥1.70 respektive ≥2.00 registerades som sannolika identifieringar på species respektive genus nivå.
- I studien användes endast kliniska blodprover för korttids-odling.

Totalpoäng: 1

6 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?
Välj ett alternativ:

- Av de fem *Staphylococcus epidermidis* proverna kunde endast fyra detekteras på på speciesnivå efter 2 timmars inkubation.
- En av de två *Streptococcus pyogenes* proverna kunde detekteras på speciesnivå efter 24 timmar.
- Av de simulerade gram negativa blodproverna kunde 77 av 84 prover korrekt identifieras på speciesnivå.
- Samtliga (120/120) korttids-odlingar av gram-positiva bakterier kunde identifieras på speciesnivå med hjälp av MALDI-ToF MS.
- En högre andel gram positiva bakterier än gram negativa bakterier kunde identifieras på speciesnivå.

Totalpoäng: 1

7 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?
Välj ett alternativ:

- Efter 2 timmars inkubation kunde en större andel (94.8%) av samtliga bakterier i de kliniska proverna identifieras.
- Inkubationstiden hade ingen påverkan på identifiering av *Providencia rettgeri* i de kliniska proverna.
- Samtliga Klebsiella species i de kliniska proverna kunde identifieras på speciesnivå efter 2 timmars inkubation.
- I de kliniska proverna kunde en större andel *Staphylococcus aureus* identifieras på species nivå efter 2 timmars inkubation i jämförelse med *Staphylococcus epidermidis*.
- I de kliniska proven kunde *Staphylococcus pasteuri* detekteras på både genus och speciesnivå efter 2 timmars inkubation.

Totalpoäng: 1

8 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?
Välj ett alternativ:

- Det mest frekventa grampositiva bakterierna som identifierades i de kliniska proverna var stafylokocker.
- I de kliniska proven kunde *Streptococcus dysgalactiae* detekteras på både genus och speciesnivå efter 2 timmars inkubation.
- Efter 4 timmars inkubation kunde 86.2% av de grampositiva bakterierna identifieras på speciesnivå i de kliniska proverna.
- Det var ingen skillnad i detektionsnivå på species respektive genusnivå efter 2 timmars inkubation i de kliniska proverna.
- Inkubationstiden hade ingen betydelse för detektion av kliniskt relevanta gram positiva bakterier på genus nivå.

Totalpoäng: 1

9 Läs bifogad artikel och besvara följande fråga:

Vilket av följande påståenden är **MEST KORREKT** ?

Välj ett alternativ:

- Gram negativa bakterier är svårare att identifiera än gram positiva bakterier med MALDI-ToF MS.
- Till skillnad från tidigare studier sågs i denna en korrelation mellan temperatur och transporttid.
- Att det var lättare att identifiera bakterier i de kliniska proverna beror troligen på att en del av patienterna tagit antibiotika.
- Förlängd inkubation i rumstemperatur hade en negativ effekt på tillväxten av *Streptococcus pyogenes.*
- Skillnaden i resultat mellan simulerade och kliniska prover efter 2 timmars inkubation beror på att de kliniska proverna troligen har ett högre antal CFU/mL.

Totalpoäng: 1

10 Läs bifogad artikel och besvara följande fråga:

Vilket av följande alternativ är **MEST KORREKT**?

Välj ett alternativ:

- Korttids-blododling följt av MALDI-ToF är en tillförlitlig metod och varierar marginellt mellan gram negativa och grampositiva bakterier.
- Korttids-blododling följt av MALDI-ToF är en tillförlitlig metod och påverkas inte av transporttemperaturen.
- Korttids-blododling följt av MALDI-ToF är en tillförlitlig metod och påverkas inte av vilka mikroorganismer som identifieras.
- Korttids-blododling följt av MALDI-ToF är en tillförlitlig metod och påverkas inte av förlängd transporttid.
- Korttids-blododling följt av MALDI-ToF är en tillförlitlig metod och varierar marginellt mellan patienter.

Totalpoäng: 1

Question 10
Attached

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

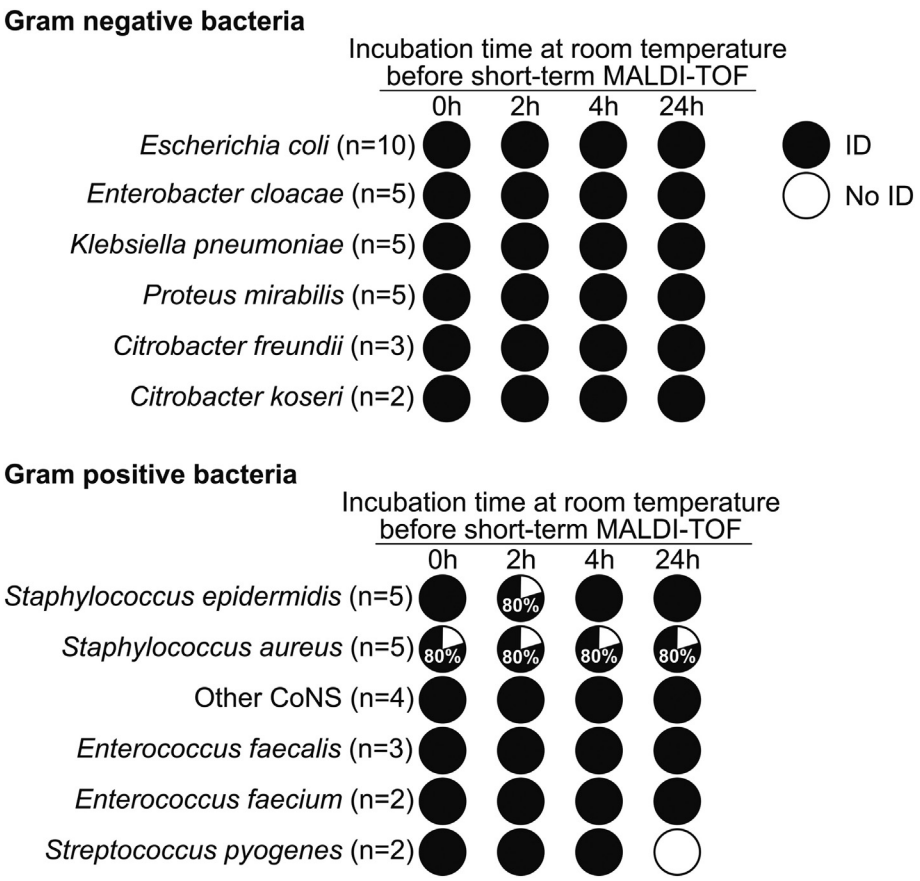

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

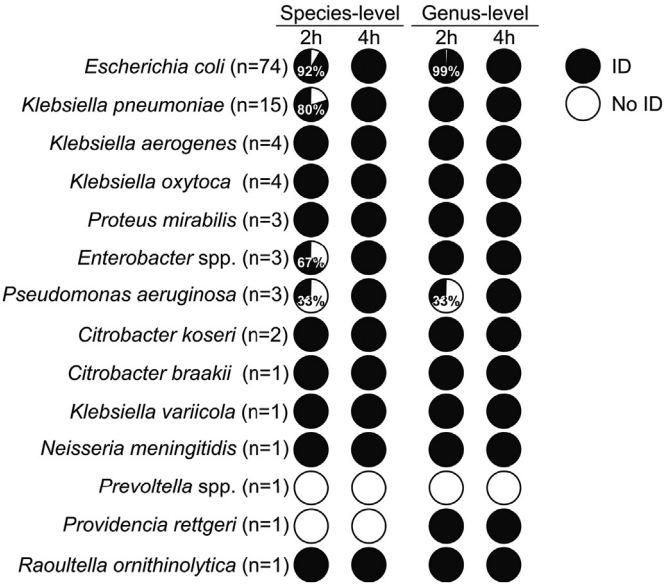

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

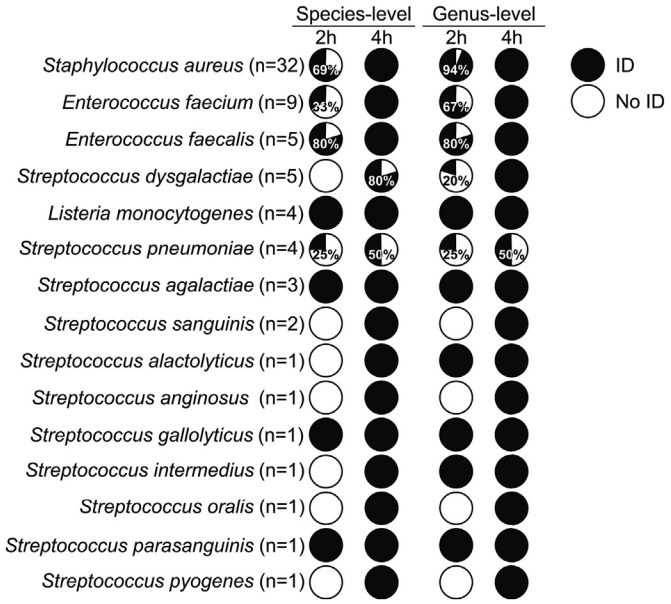

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

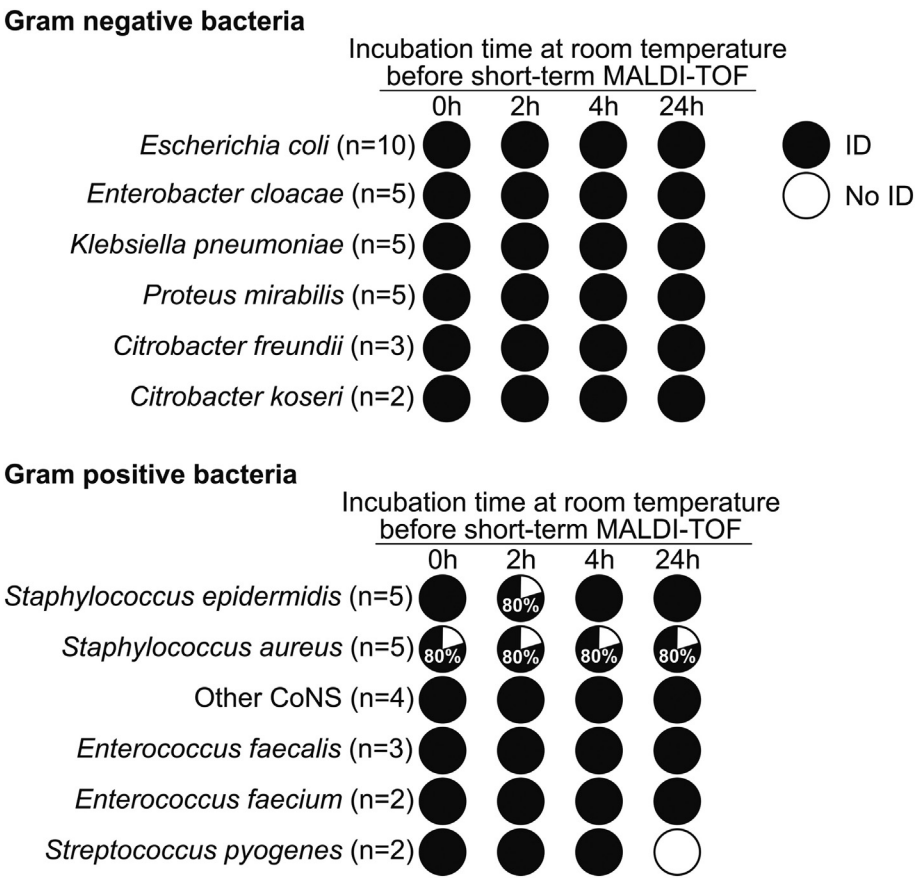

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

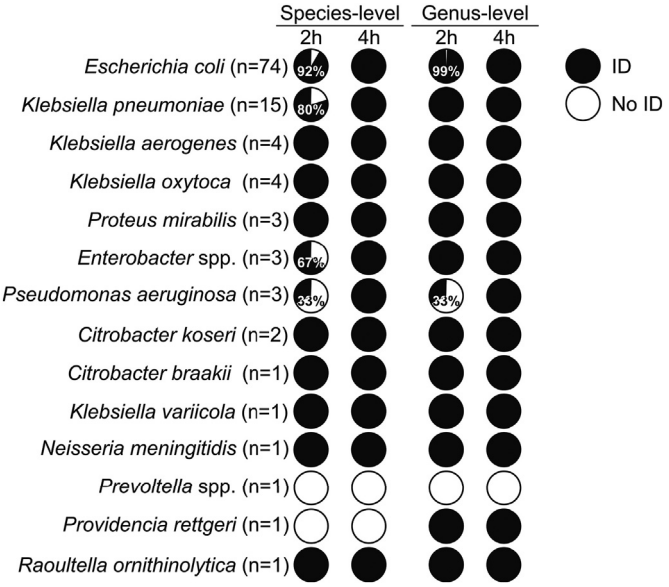

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

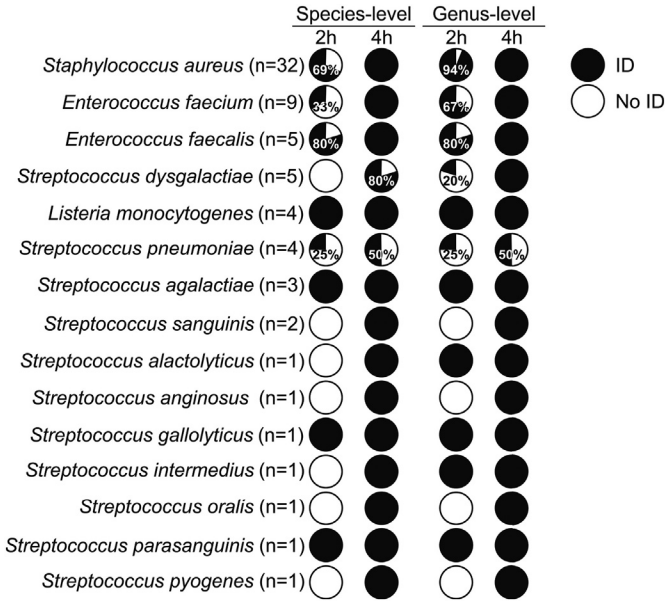

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

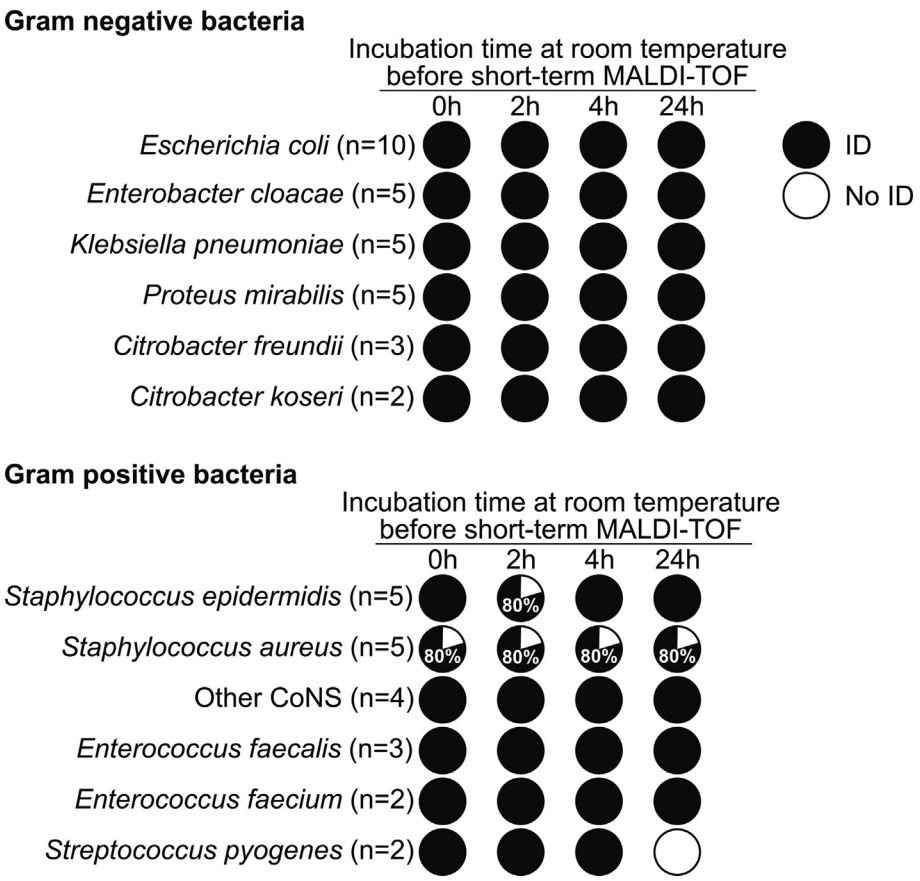

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

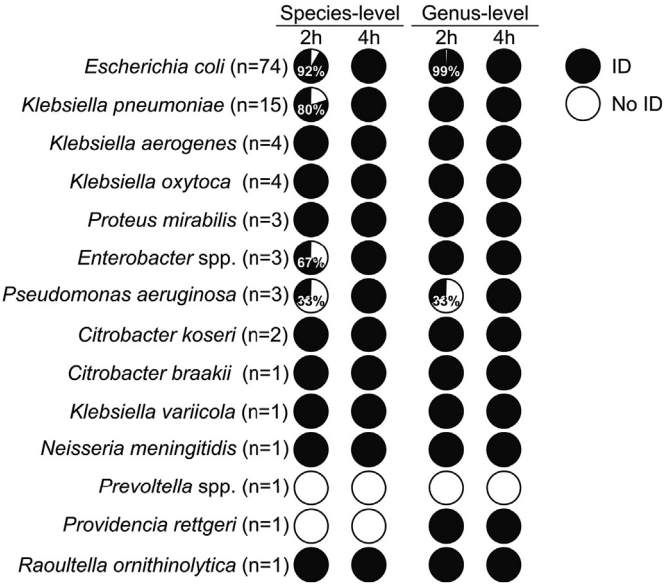

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

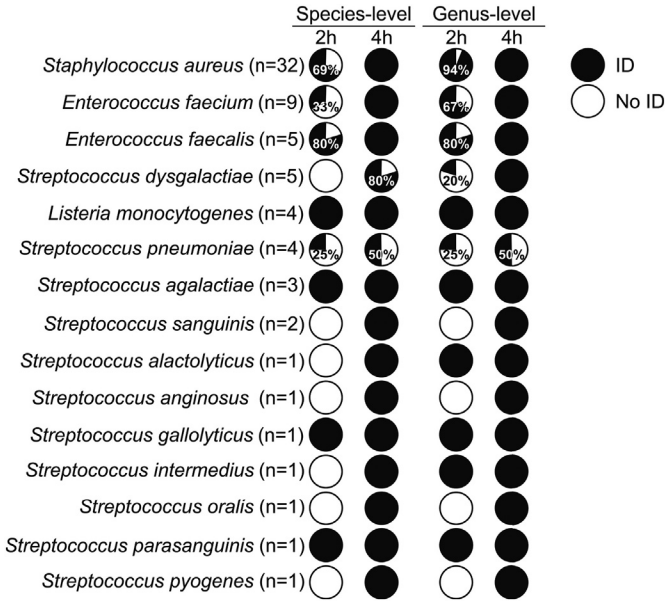

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

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Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

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Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

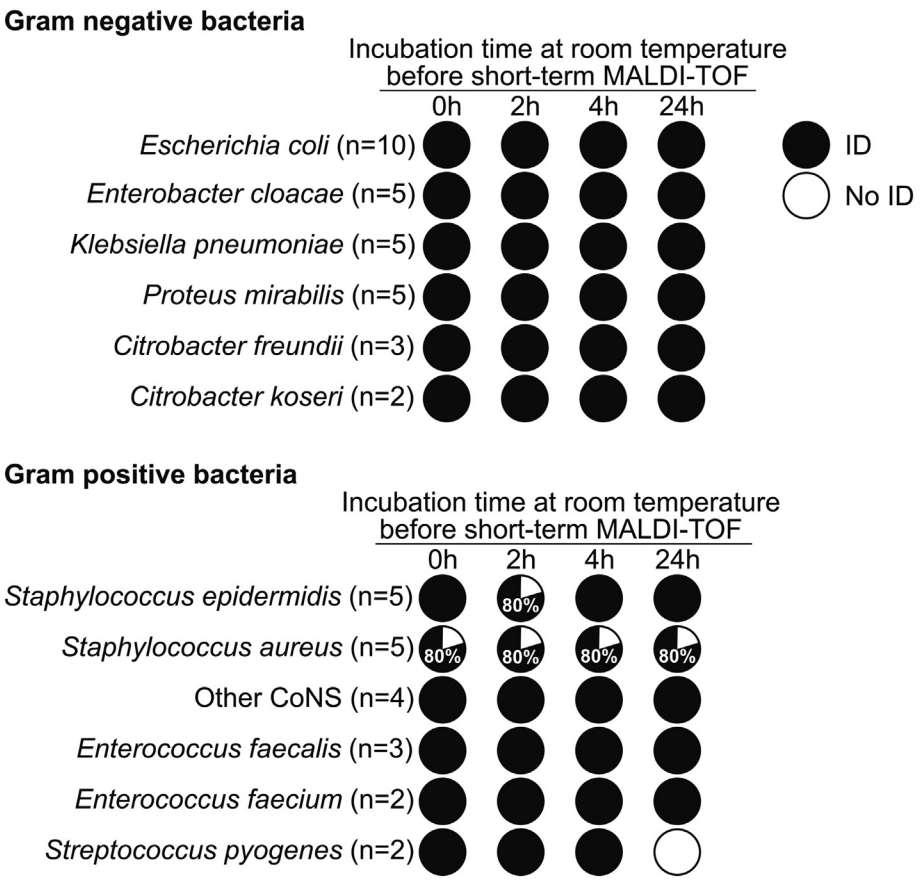

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

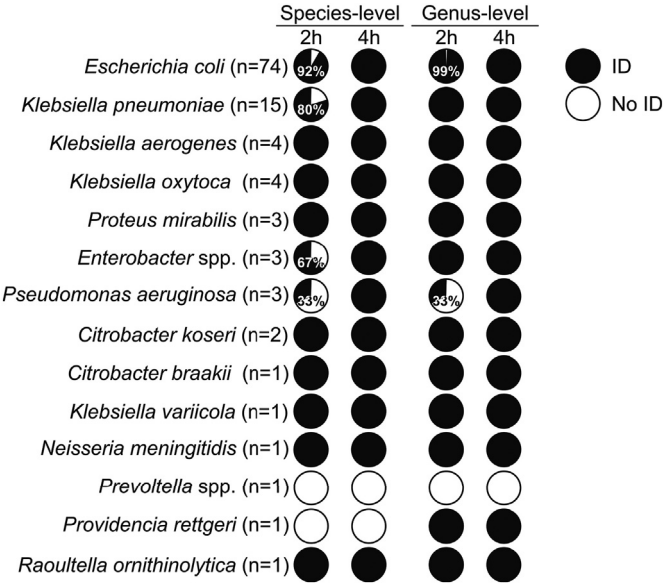

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

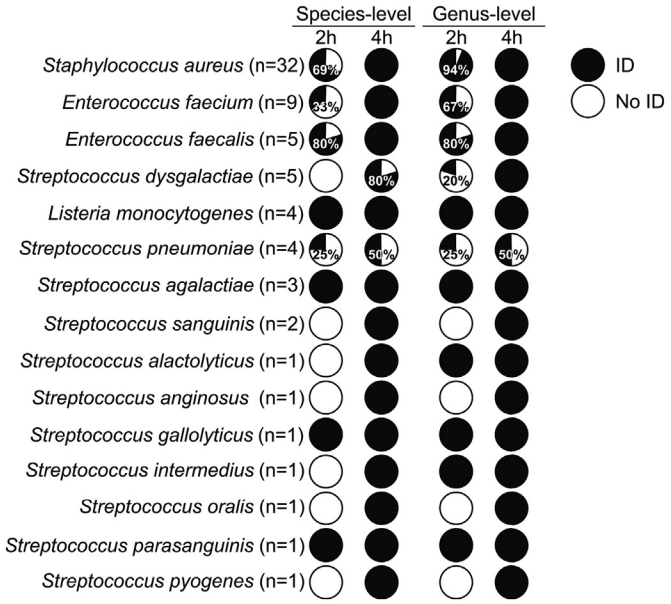

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

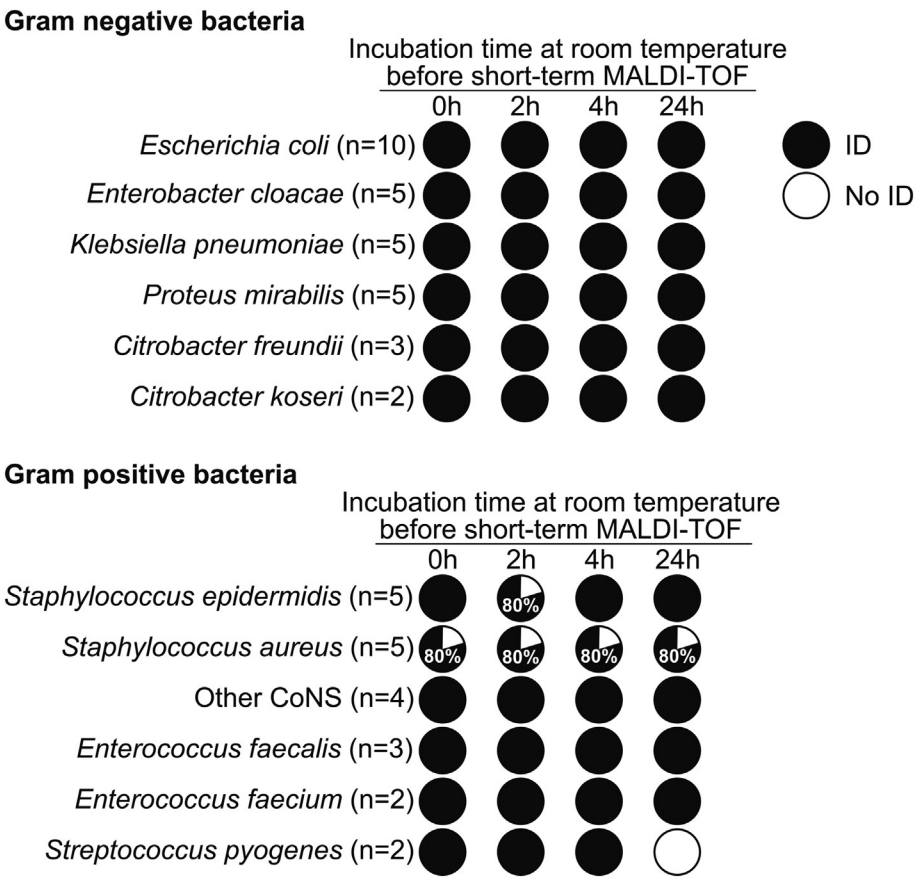

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

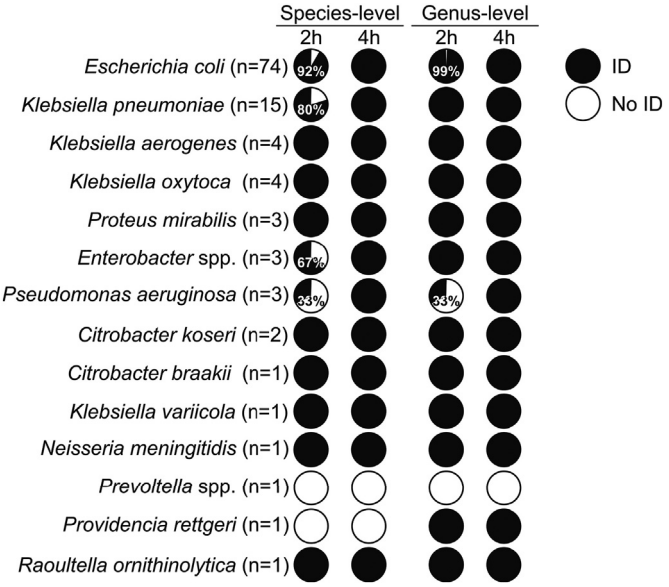

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

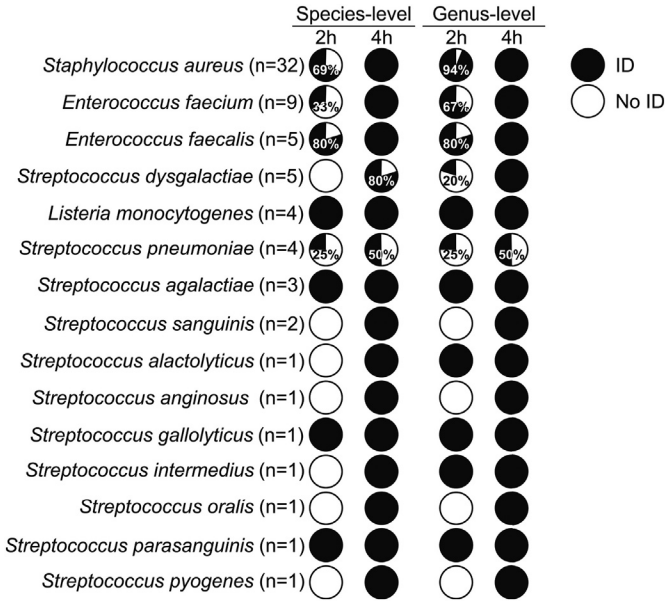

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

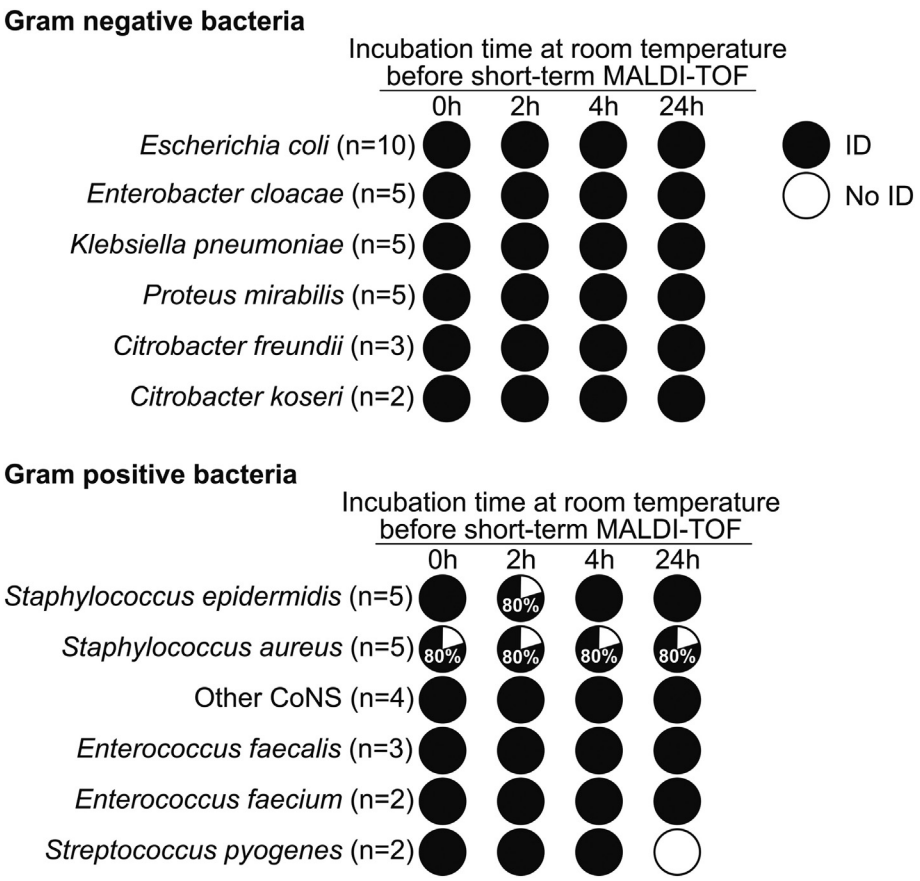

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

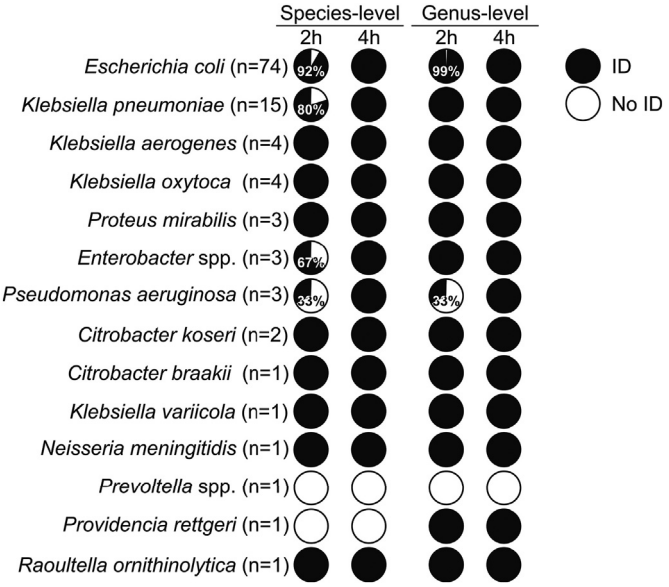

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

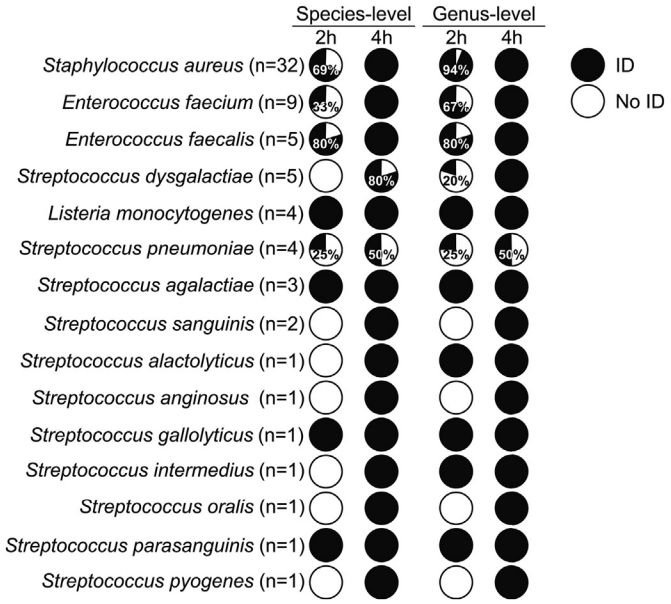

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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We would like to thank the laboratory personnel at the blood culture lab of Karolinska University Hospital, Huddinge for their technical assistance with this study.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

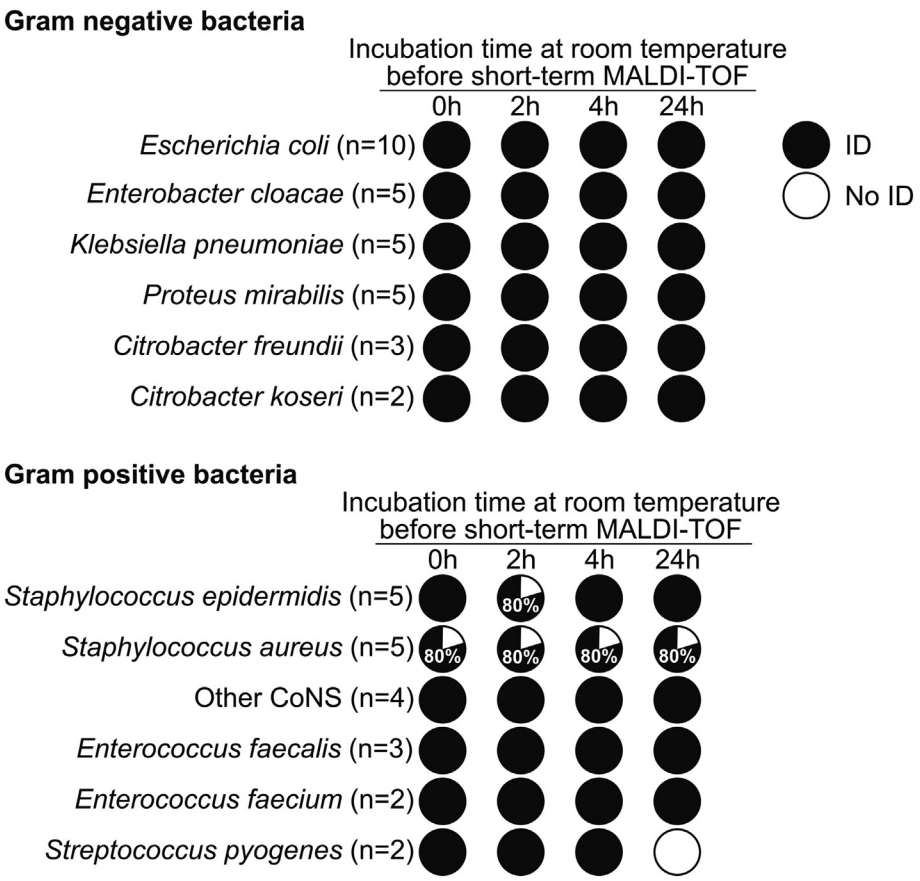


Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

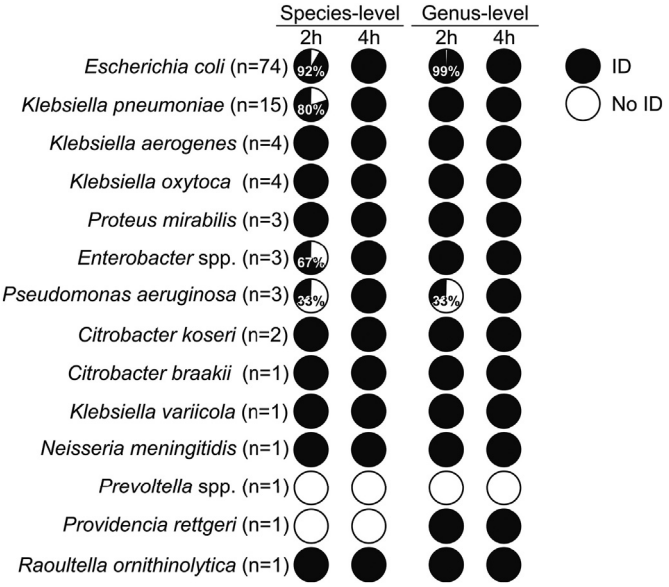


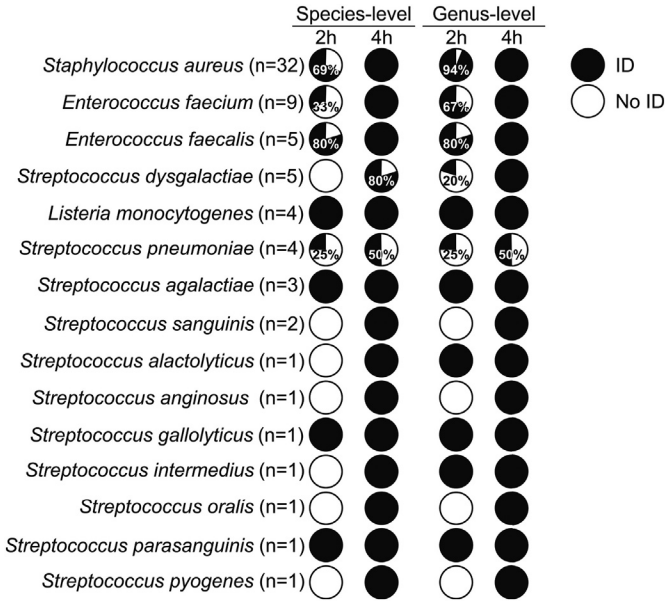
Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)



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The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

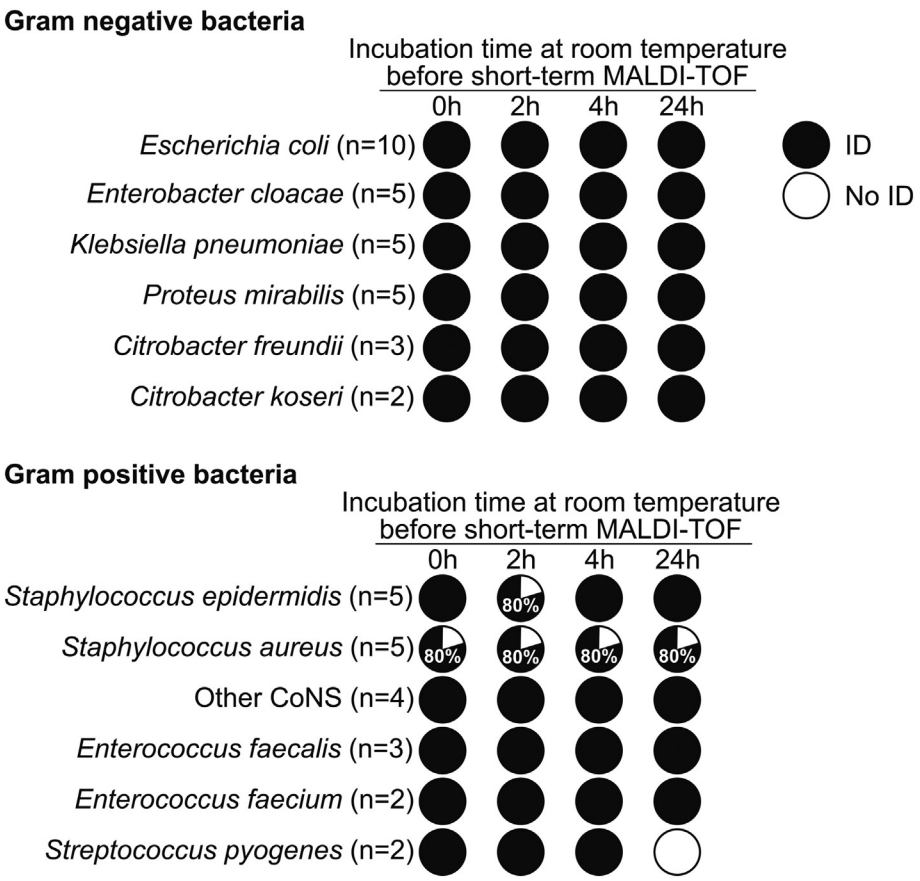

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

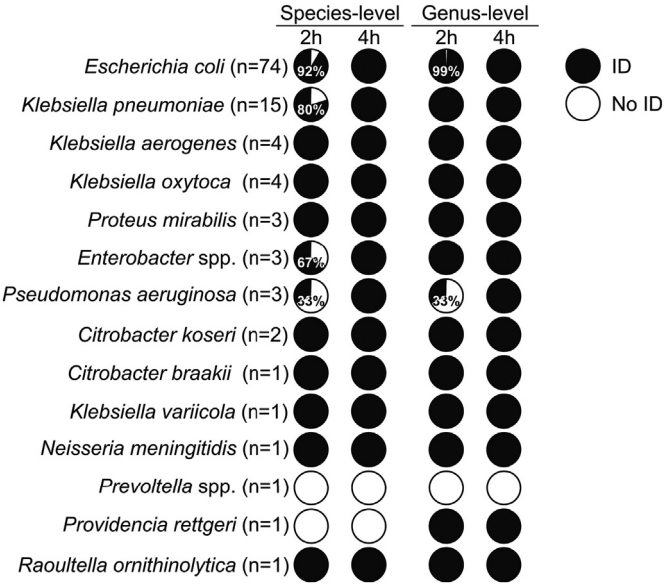

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

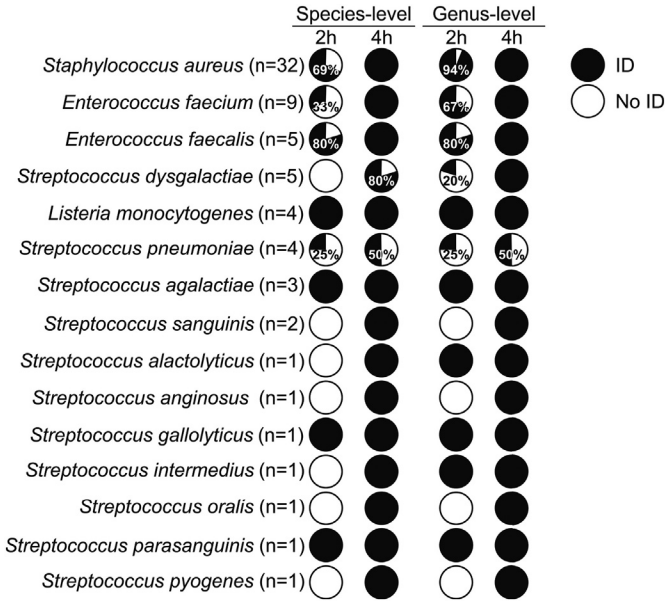

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

Materials/methods: To simulate the effect of transportation and delayed analysis of blood culture bottles, 51 simulated blood culture bottles were incubated for 0, 2, 4 and 24 h at room temperature. After each time interval, a 2 to 4 h short-term culture followed by MALDI-TOF MS was performed. In addition, 257 prospective clinical positive blood culture bottles were analysed with the same method after a 24 h incubation at room temperature.

Results: In simulated samples, all (120/120) Gram-negative bacteria and 77/84 (91.6%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture, regardless of the duration of simulated transport time. In the clinical samples, 100/116 (86.2%) Gram-negative, and 44/141 (31.2%) Gram-positive bacteria were accurately identified at species-level after a 2 h short-term culture. After contaminants were excluded, 39/71 (54.9%) Gram-positive bacteria could be identified after 2 h. After a 4 h short-term culture, 112/116 (96.6%) Gram-negative, and 108/141 (76.6%) Gram-positive bacteria were accurately identified at species-level. Of the clinically relevant Gram-positive bacteria, 68/71 (95.8%) were identified at species-level after 4 h.

Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

Blood cultures are the gold standard in detection of microorganisms in patient bloodstream infections. One of the major disadvantages of blood cultures is long turn-around time from sampling to identification of microorganisms causing bloodstream infections.

Recent developments enabled reliable rapid identification of microorganisms from positive blood cultures (Özenci et al., 2018; Miller et al., 2018a). Previously, we and others presented a novel method for rapid and reliable identification of microorganisms from blood culture bottles based on simple routine diagnostic tests following a decreased incubation time on a solid medium (Idelevich et al., 2014). Currently, among many recent methods available, short-term culture followed by MALDI-TOF MS is one of the most common methods for fast identification of microorganisms from blood cultures (Altun et al., 2015). Recently it was reported that 40% of the blood culture labs in Europe implemented the method in clinical routine (Idelevich et al., 2019). The

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method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture.

Transport time of blood culture bottles to the laboratories has been one of the major problems causing unnecessary delay in total turn-around time. Previously we showed that the transport times of blood culture bottles from tertiary care hospitals to the central laboratory ranged between 3 and 15 h (Rönnberg et al., 2013). With the improvements in blood culture systems of software, many central laboratories establish so called satellite blood culture systems and place them in clinics and other hospitals where the blood culture samples were taken. Blood culture bottles can therefore be incubated immediately after sampling. However, the positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. Since the success of short-term culture followed by MALDI-TOF MS is probably dependent on the growth phase of microorganisms in the blood culture bottle, the outcome by the method may be negatively affected by delayed analysis of the bottles.

The performance of rapid identification methods after transport at room temperature has not yet been studied previously. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

2. Materials and methods

2.1. Blood culture-bottles and –system

Blood samples were collected by standard protocols at Karolinska University Hospital in BacT/Alert-FA Plus and BacT/Alert-FN Plus blood culture bottles (BioMérieux, Durham, NC, USA) as previously described (Yu et al., 2020). Simulated bottles were incubated in the Bact/Alert® 3D system and the clinical samples in BacT/Alert Virtuo automated blood culture system (BioMérieux, Durham, NC, USA) until they turn positive, or for a maximum incubation time of 5 days. Only one positive bottle per patient was analysed. Bottles yielding polymicrobial growth or fungal growth were excluded.

2.2. Bacterial samples

The simulated blood cultures were prepared using clinical isolates. 51 clinical isolates collected between December 2018 and April 2019 from positive blood cultures were included in this study. The species were selected due to their frequent occurrence in bloodstream infection patients at our centre and consisted of six of each of the most common Gram-negative bacteria and Gram-positive bacteria. Table 1 summarizes the bacteria included in the study. The isolates were stored at −80 °C. Isolates were subcultured onto blood agar plates and incubated at 37 °C for 18 h for use in simulated blood cultures.

Table 1
Bacteria that were used in the simulated blood culture bottles.

Species	Number
Escherichia coli	10
Klebsiella pneumoniae	5
Citrobacter freundii	3
Citrobacter koseri	2
Enterobacter cloacae	5
Proteus mirabilis	5
Total Gram-negative bacteria	**30**
Staphylococcus aureus	5
Staphylococcus epidermidis	5
Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

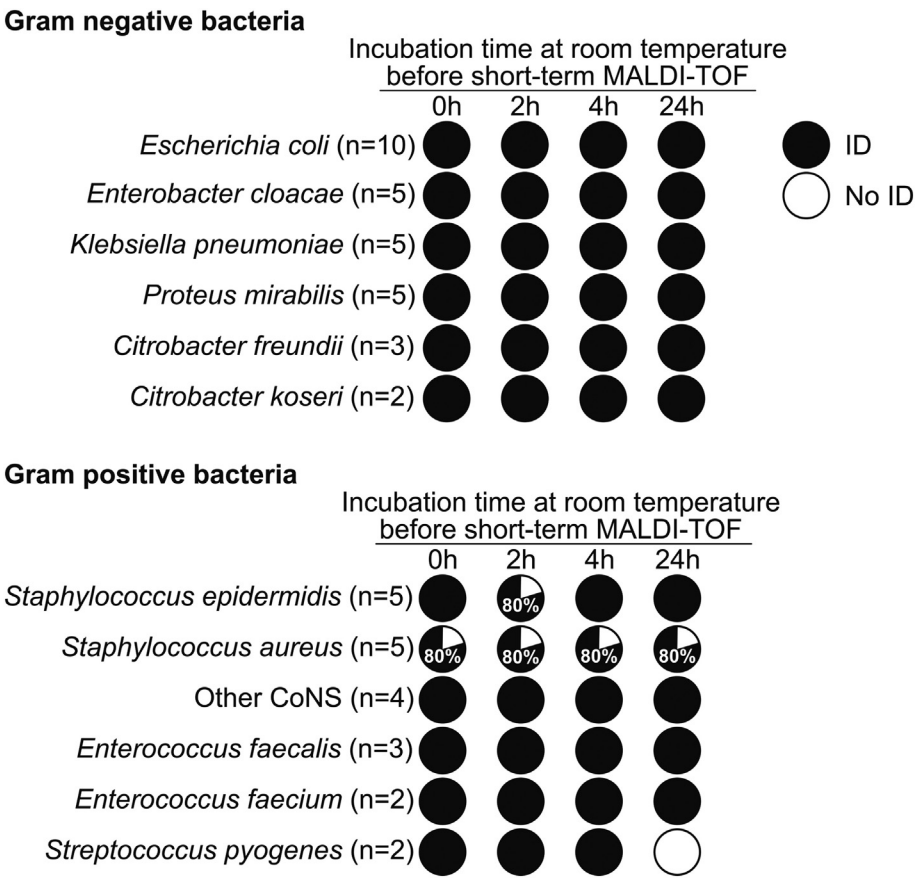

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

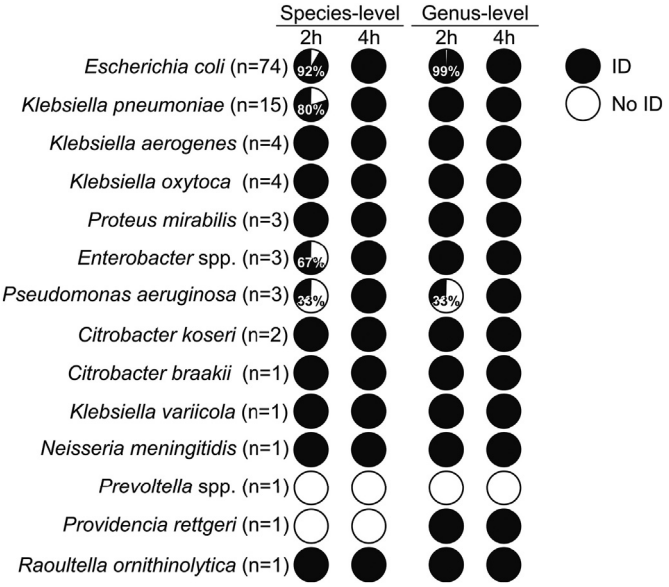

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

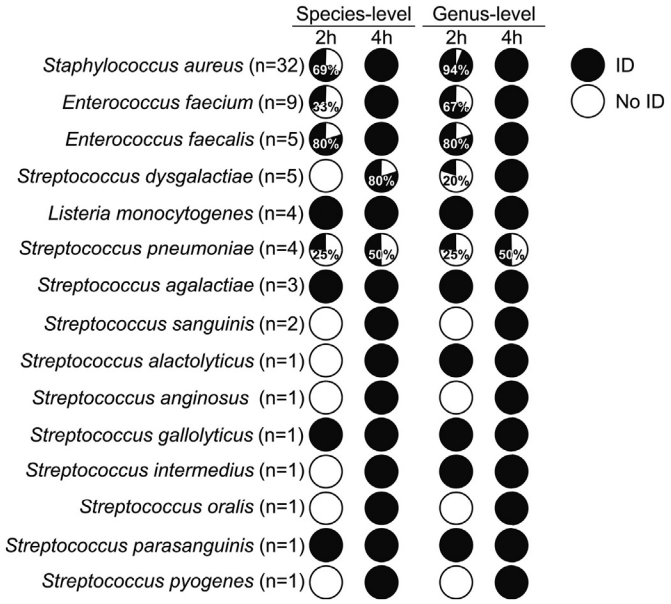

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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The impact of delayed analysis of positive blood cultures on the performance of short-term culture followed by MALDI-TOF MS

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ABSTRACT

Background: Short-term culture followed by MALDI-TOF MS is one of the most widely used methods for fast identification of microorganisms from blood cultures. The method identifies the vast majority of bloodstream infection pathogens in 2–6 h after positive blood culture. Transport time of blood culture bottles to laboratories is a major problem affecting total turnaround time. Therefore, many central laboratories establish satellite blood culture systems in other clinics and hospitals to allow blood culture bottles to be incubated immediately after sampling. However, positive blood culture bottles still need to be transported to the clinical microbiology laboratory for analysis. The aim of this study was to investigate how delayed analysis of positive blood culture bottles would affect the short-term culture followed by MALDI-TOF MS method.

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Conclusions: Short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

1. Introduction

Early initiation of appropriate antibiotic therapy is crucial in reducing mortality, and morbidity rates in patients with bloodstream infections (Judd et al., 2014). Rapid and accurate detection and identification of microorganisms in bloodstream infections has shown to be decisive in early appropriate antimicrobial therapy.

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Enterococcus faecalis	3
Enterococcus faecium	2
Group A *streptococci* (*Streptococcus pyogenes*)	2
Other coagulase negative *staphylococci*	4
Total Gram-positive bacteria	**21**
Total overall	**51**

2.3. Simulated blood culture bottles

The cultured bacteria were suspended in sterile 0.9% NaCl to a turbidity of 0.5 McFarland (1.5×10^8 CFU/ml) and diluted to a final concentration of 15,000 CFU/ml. 100 CFU (7 µl) from the suspension was added to 5 ml sterile human blood (obtained from Transfusion medicine, Karolinska University Hospital, Huddinge) and inoculated in a BacT/Alert-FA Plus bottle. The bottles were incubated in the Bact/Alert® 3D system and removed after signaling positive. The bacterial suspension was also cultured on three blood agar plates [Columbia Blood Agar Base 43.0 g (Alpha Biosciences, Baltimore, Maryland, USA), L-tryptophan 0.1 g (Merck Millipore, Burlington, Massachusetts, USA), distilled water 1000 ml and defibrinated horse blood 50 ml, pH 7.3 ± 0.2 (Håtunalab AB, Bro, Sweden), obtained from Substrate Unit, Clinical Microbiology, The Karolinska University Laboratory, Huddinge, Sweden)] as an inoculation CFU control, showing no major discrepancy.

2.4. Clinical blood culture bottles

In total, 257 blood culture bottles that were collected from patients with suspected bloodstream infection and sent to the laboratory were prospectively analysed. The clinical samples were collected between June 2019 and September 2019. Of these bottles, 48 were anaerobic BacT/Alert-FN Plus and 209 aerobic BacT/Alert-FA Plus.

2.5. Short-term culture

Simulated blood culture bottles were cultured after being incubated for 0, 2, 4 and 24 h at room temperature after blood cultures signal positive to imitate transport time to the laboratory. Clinical blood culture bottles that turned positive in the blood culture system were prospectively analysed after 24 h in room temperature to imitate long transport time.

Short-term cultures were performed on both simulated and clinical blood culture bottles prior to analysis with MALDI-TOF MS by culturing 10 drops of blood culture broth on blood agar. The agar plates were incubated at 37 °C in 5% CO_2 atmosphere for 2 h and thereafter were analysed with MALDI-TOF MS. If no growth was visible after 2 h, the agar plates were incubated for a total of 4 h. Hence, in total 204 short-term cultures were performed from the 51 simulated samples.

2.6. MALDI-TOF MS

Following the short-term culture, identification was performed with the MALDI-TOF MS microflex LT/SH system (Bruker Daltonik) along with the software Bruker Biotyper 3.1 (version 4613; Bruker Daltonik). The samples were analysed in duplicates. MALDI-TOF MS scores ≥1.70 and ≥2.00 were accepted as successful identifications at genus and species level, respectively, as recommended in criteria for data interpretation set by the manufacturer.

3. Results

3.1. Simulated blood culture bottles

In simulated samples, the short-term culture followed by MALDI-TOF MS successfully identified 197/204 (96.5%) and all (204/204) microorganisms at species and genus level, respectively. All (120/120) of the short-term cultures of Gram-negative bacteria were correctly identified with MALDI-TOF MS at species level (Fig. 1), consistently providing score-values > 2.00. In contrast, 77/84 (91.6%) of the short-term cultures of Gram-positive bacteria were correctly identified with

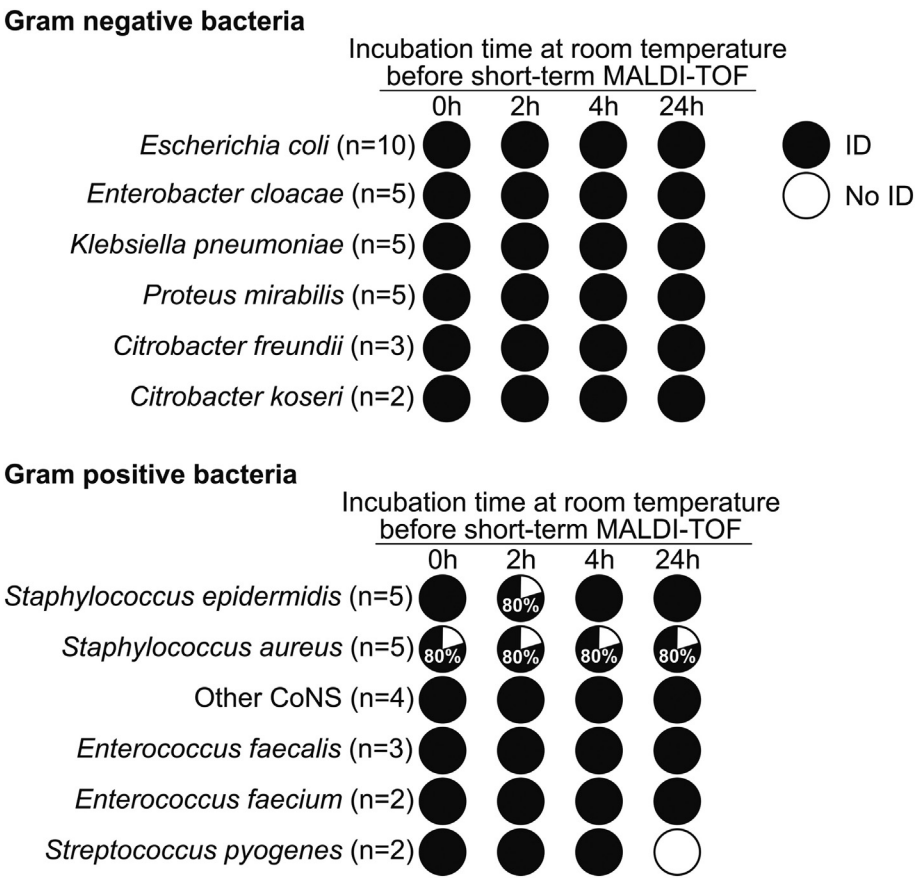

Fig. 1. Identification of bacteria at species level in simulated samples after different time intervals following positive blood culture. Percentage of simulated samples per bacteria strain that were identified at species level using 2 h short-term culture and MALDI-TOF MS, after incubation at 0, 2, 4, and 24 h at room temperature following blood cultures turning positive.

MALDI-TOF MS at species level. Four of the short-term cultures only identified bacteria at genus level. All of those belonged to the same sample and the bacteria was *Staphylococcus aureus*. The other short-term cultures only identifiable at genus level were one *Staphylococcus epidermidis* and the two *Streptococcus pyogenes* samples following the 24 h incubation of the blood culture bottle (Fig. 1).

3.2. Clinical blood culture bottles

In total, 257 (209 BacT/Alert-FA Plus, and 48 BacT/Alert-FN Plus) positive clinical blood culture bottles were studied in order to analyse the performance of the method. Short-term culture MALDI-TOF MS method accurately identified microorganisms at species-level in 144/257 (56.0%) blood culture bottles after 24 h of incubation at room temperature. 100/116 (86.2%) of these bacteria were Gram-negative bacteria (Fig. 2), and 44/141 (31.2%) were Gram-positive bacteria (Table 2). Identification at genus-level after 2 h of incubation time were observed in 189/257 (73.5%) of all samples, with 110/116 (94.8%) being Gram-negative bacteria (Fig. 2), and 79/141 (56.0%) being Gram-positive bacteria (Table 2).

Overall, accurate identification at species-level following the 4 h incubation were observed in 220/257 (85.6%) of all samples, of which 112/116 (96.6%) were Gram-negative bacteria, and 108/141 (76.6%) Gram-positive bacteria. Identification at genus-level after 4 h of incubation time was obtained in 243/257 (94.5%) of all samples, with 113/116 (97.4%) Gram-negative bacteria, and 130/141 (92.2%) Gram-positive bacteria. The performance of the method in clinically relevant Gram-positive bacteria is presented in Fig. 3. After a 2 h incubation, 39/71 (54.9%) and 53/71 (74.6%) of clinically relevant Gram-positive bacteria could be identified at species- and genus-level, respectively. The 4 h incubation yielded accurate identification in 68/71 (95.8%) and 69/71 (97.2%) at species and genus-level, respectively (Fig. 3).

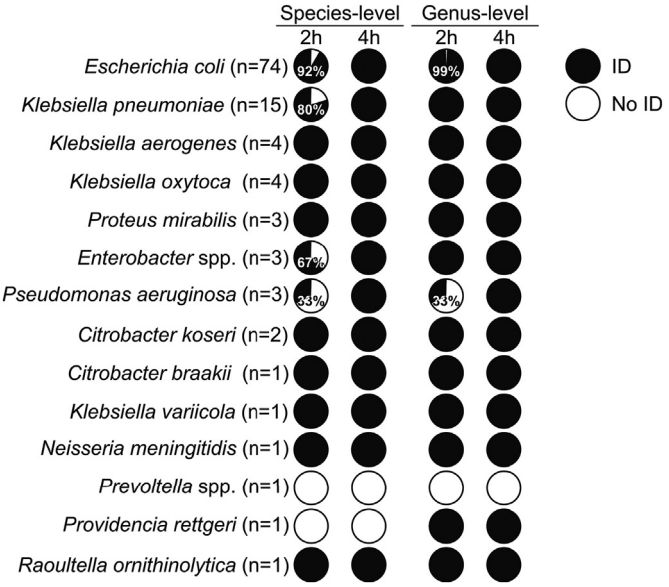

Fig. 2. Identification of Gram-negative bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of Gram-negative bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

4. Discussion

The increased health care costs have led to centralization of clinical laboratories. This has resulted in a significant challenge for timely

Table 2
Identification of Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS.

Species	ID after 2 h of incubation		ID after 4 h of incubation		Total
	Species-level	Genus-level	Species-level	Genus-level	
Staphylococcus aureus	22	30	32	32	32
Staphylococcus epidermidis	1	8	14	28	32
Staphylococcus hominis	1	7	12	15	16
Enterococcus faecium	3	6	9	9	9
Staphylococcus capitis	1	3	6	7	9
Enterococcus faecalis	4	4	5	5	5
Streptococcus dysgalactiae	0	1	4	5	5
Listeria monocytogenes	4	4	4	4	4
Streptococcus pneumoniae	1	1	2	2	4
Staphylococcus lugdunensis	1	2	3	3	3
Streptococcus agalactiae	3	3	3	3	3
Bacillus cereus	0	1	2	2	2
Staphylococcus canis	1	2	2	2	2
Streptococcus sanguinis	0	0	2	2	2
Cutibacterium spp.	0	0	0	0	1
Micrococcus luteus	0	1	0	1	1
Rothia mucilaginosa	0	1	0	1	1
Staphylococcus haemolyticus	0	0	0	1	1
Staphylococcus pasteuri	0	1	1	1	1
Staphylococcus pettenkoferi	0	0	0	0	1
Streptococcus alactolyticus	0	1	1	1	1
Streptococcus anginosus	0	0	1	1	1
Streptococcus gallolyticus	1	1	1	1	1
Streptococcus intermedius	0	1	1	1	1
Streptococcus oralis	0	0	1	1	1
Streptococcus parasanguinis	1	1	1	1	1
Streptococcus pyogenes	0	0	1	1	1
Total Gram-positive bacteria	44 (31.2%)	79 (56.0%)	108 (76.6%)	130 (92.2%)	141 (100%)
Total bacteria overall	144 (56.0%)	189 (73.5%)	220 (85.6%)	243 (94.6%)	257 (100%)

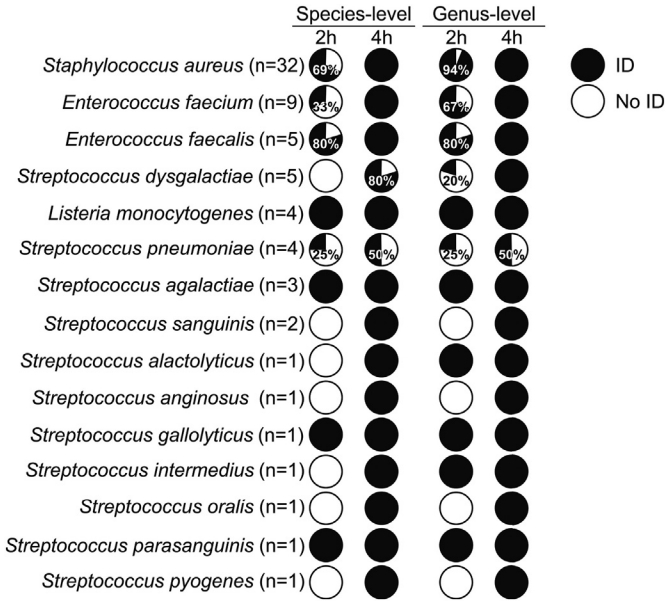

Fig. 3. Identification of clinically relevant Gram-positive bacteria in clinical samples after 24 h at room temperature using short-term culture followed by MALDI-TOF MS. Percentage of clinically relevant Gram-positive bacteria in prospective clinical blood cultures that were identified at species- and genus-level using short-term culture and MALDI-TOF MS, after incubation 24 h at room temperature following positive blood culture.

transportation of blood cultures, i.e., between sample collection and incubation in a blood culture system. It is considered that optimal transport time for blood cultures is < 2 h (Willems et al., 2012; Miller et al., 2018b). However, two previous studies from two different countries showed that the overall median transport time for blood culture was as long as 9 and 10 h, respectively (Rönnberg et al., 2013; Kerremans et al., 2009). The improvement in blood culture systems have opened the possibility for establishment of systems that are placed in the hospitals where the samples are taken with 24/7 activity. This has become an effective and common solution for dramatic time delay with transportation of blood cultures to central laboratories (Rönnberg et al., 2013). However, the blood culture bottles that signal positive in the system should still be transported to the central microbiology laboratory. In the current study, the transport times of positive blood culture bottles were not studied. However, it is reasonable to suggest that the median time is seldom < 2 h, especially for blood culture systems that are located in other hospitals than the central laboratory. The aim of this study was to investigate the effect of delayed analysis of positive blood culture bottles by short-term culture followed by MALDI-TOF MS.

We observed that the method had high performance in simulated blood culture bottles, being able to provide accurate identification at species level for 197/204 (96.6%) of all bacteria analysed after only 2 h of incubation on solid media. All 7 bacteria that could not be identified were Gram-positive, suggesting that Gram-positive bacteria generally are more difficult to identify than Gram-negative bacteria as supported by previous other studies (Idelevich et al., 2014; Altun et al., 2015; Curtoni et al., 2017). All Gram-negative bacteria samples were accurately identified at species level after each time interval and the time interval did not significantly affect the score-values for the Gram-negative bacteria. Hence, the growth abilities of the Gram-negative bacteria included in this study does not seem to be affected by a 24 h delayed analysis of blood culture bottles after removal from the culture system. MALDI-TOF MS scores of ≥2.00 is accepted as reliable identification at species level. The only Gram-positive bacteria that had a decrease in score-values following prolonged incubation in room temperature were *S. pyogenes*, where both samples received score-values below 2.00 after 24 h.

The simulated samples are per definition artificial and might

theoretically not reflect the clinical picture fully. Therefore, we analysed clinical samples with the worst-case delayed transport time scenario where the samples incubated at room temperature after turning positive in the blood culture system. Interestingly, the majority of Gram-negative bacteria (86%) could be identified at species level after 2 h short-term culture whereas only 31% of the Gram-positive bacteria could be identified at this time point. After 4 h short-term culture, 96% of Gram-negative bacteria and 76% Gram-positive bacteria were accurately identified at species-level. When the data for clinically relevant Gram-positive bacteria were analysed separately we observed that 96% of the Gram-positive bacteria could be identified at species-level after 4 h short-term culture. The underlying reason for the different results between the simulated bottles and the prospective clinical samples for samples analysed after 2 h short-term culture is not known. It is plausible to suggest that the low start concentration and/or the presence of antibiotics in clinical samples might play a role. Previous studies showed that the bacterial concentration in patients' blood varies a lot and is seldom > 100 CFU/ml [Reviewed in (Lamy et al., 2016)]

The success rate of the prospective clinical samples in the present study concords with previous studies regarding short-term cultures from clinical prospective blood cultures compared to the simulated samples. However, it should be noted that the previous studies about short-term cultures have been made at the time blood culture bottles turn positive. In the present study, the samples were incubated for 24 h in room temperature.

Several studies have explored the optimal temperature for transport or pre-incubation of blood culture bottles prior to entry into the blood culture system (Sautter et al., 2006; Wilms et al., 2009) and recommended that room temperature should be used in transporting blood culture bottles. However, none of the previous studies have explored if there was an effect of temperature on delayed analysis of blood culture bottles after they have signalled positive. The present study shows that the present method has high performance in blood culture bottles transported at room temperature, hence the short-term culture method can reliably be used in the clinical routine even for the blood culture bottles with long transport time after blood culture turn positive in the satellite blood culture systems.

Laboratory diagnosis of bloodstream infections is complicated and can be affected by several factors, including sampling, transport time to blood culture systems, handling of positive blood culture bottles in the laboratory, and even the post-analytical phase. To our knowledge, there is no prior study analysing the effect of transportation of positive blood cultures and downstream performance of identification methods, therefore this is the first study focusing on the potential impact of delayed analysis of the positive blood culture bottles. In conclusion, short-term culture followed by MALDI-TOF MS can provide fast and accurate results for identification of clinically relevant bacteria, despite long transportation times from satellite laboratories. The present data shows that the method can be used for identification of microorganisms from positive blood cultures transported from satellite blood culture systems.

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