**Work Flow Analysis of Around-the-clock Processing of Blood Culture Samples and Integrated MALDI-TOF Mass Spectrometry Analysis for the Diagnosis of Bloodstream Infections**

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**BACKGROUND:** Because sepsis has a high mortality rate, rapid microbiological diagnosis is required to enable efficient therapy. The effectiveness of MALDI-TOF mass spectrometry (MALDI-TOF MS) analysis in reducing turnaround times (TATs) for blood culture (BC) pathogen identification when available in a 24-h hospital setting has not been determined.

**METHODS:** On the basis of data from a total number of 912 positive BCs collected within 140 consecutive days and work flow analyses of laboratory diagnostics, we evaluated different models to assess the TATs for batch-wise and for immediate response (real-time) MALDI-TOF MS pathogen identification of positive BC results during the night shifts. The results were compared to TATs from routine BC processing and biochemical identification performed during regular working hours.

**RESULTS:** Continuous BC incubation together with batch-wise MALDI-TOF MS analysis enabled significant reductions of up to 58.7 h in the mean TATs for the reporting of the bacterial species. The TAT of batch-wise MALDI-TOF MS analysis was inferior by a mean of 4.9 h when compared to the model of the immediate work flow under ideal conditions with no constraints in staff availability.

**CONCLUSIONS:** Together with continuous cultivation of BC, the 24-h availability of MALDI-TOF MS can reduce the TAT for microbial pathogen identification within a routine clinical laboratory setting. Batch-wise testing of positive BC loses a few hours compared to real-time identification but is still far superior to classical BC processing. Larger prospective studies are required to evaluate the contribution of rapid around-the-clock pathogen identification to medical decision-making for septicemic patients.

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Sepsis is characterized by an inflammation affecting the whole body (systemic inflammatory response syndrome) in the presence of infection (1). The Surviving Sepsis Campaign recommends attempting to prove the presence of an infection by use of methods that include microbiological testing (2). The gold standard for detection of bloodstream infections is blood culture in liquid media (3) with subsequent cultural and biochemical identification of the culprit pathogen. Mortality from sepsis has been suggested to increase by 7% for every hour of delay in the administration of appropriate antibiotic therapy (4). Thus, rapid blood culture testing with determination of the pathogen is essential to establish a diagnosis and enable efficient therapy.

The time required for a positive blood culture result depends on the incubation time required for the initial culture of the microorganisms to turn positive, the time to positivity (TTP), and the subsequent biochemical identification, which usually takes 48 h (5). Automated blood culture systems have evolved in recent years to help reduce the incubation time until a blood culture can be identified as positive. New techniques for pathogen identification, like PCR (6-8) and fluorescence in situ hybridization (9) as well as high-throughput DNA sequencing (10), have been investigated to accelerate identification. These methods are complex and expensive and require several hours of hands-on time to deliver a result. A novel method that allows the rapid and reliable identification of most pathogenic bacteria is MALDI-TOF mass spectrometry
MALDI-TOF MS), originally established for cultured bacteria \((11, 12)\). With the use of this technology, diagnostic sensitivities and protocols for the identification of bacteria from blood culture (BC) have been reported \((13–15)\). Despite the advances in analytical technologies, a major bottleneck exists for the high proportion of blood culture samples that are collected or turn positive outside of the regular duty hours, causing long delays in the start of incubation or the further analysis of positive blood cultures. Clinical hospital laboratories usually provide in vitro diagnostic services in a 24-h setting. In the context of rapid MALDI-TOF analysis, these laboratories are therefore in a position to reduce the turnaround time (TAT) of blood culture testing, thereby assisting the clinical management of septic patients. In the present study, we examined the effect of continuous 24-h blood culture processing followed by real-time or batch-wise MALDI-TOF MS identification procedures directly from blood cultures. Our goal was to evaluate to what extent continuous incubation and 24-h MALDI-TOF MS availability would improve diagnostic outcomes under realistic emergency laboratory conditions. We compared these data with bacterial identification under idealized model conditions and also with the classical work flows of routine clinical microbiology laboratories.

### Materials and Methods

**CLINICAL STUDY SETTING**

The study was carried out in a university hospital within a metropolitan area. All blood cultures, cerebrospinal fluid, and other medical specimens requiring incubation, e.g., from patients with systemic infections, were received in an integrated laboratory reception that processed samples 24 h per day, 7 days per week (24/7). BC incubators and MALDI-TOF MS equipment were run within this 24/7 facility. BC samples that turned positive during regular duty hours (0800–1630) were routinely cultivated for subsequent biochemical identification within the microbiology department, a process that usually commenced the following day. These samples served as the control group for our study. Outside of regular duty hours, BC samples that turned positive for microbial growth were immediately processed for Gram staining and cultivated for later biochemical bacterial identification (confirmation of MALDI-TOF MS results), antibiotic susceptibility testing, and MALDI-TOF MS (Fig. 1). TATs were documented by the laboratory information system. For this setting, we analyzed the processing of 912 BC samples received within a 140-day period.

Fig. 1. Graphically illustrated comparisons of the investigated work flow concepts demonstrating the lag and processing times of all concepts and the consequences for TAT until Gram-staining and bacterial identification report.
MALDI-TOF MS bacterial identification was performed batch-wise twice per night (2200 and 0600) to be compatible with the analytical workload of the clinical chemistry emergency laboratory servicing the 1500-bed hospital. BC samples were continuously incubated on a 24/7 basis in a BACTEC 9240 BC incubator (BD) immediately upon arrival in the laboratory. BCs were continuously monitored for a maximum of 5 days according to the standard protocol of the manufacturer. MALDI-TOF MS identification was performed batch-wise for cerebrospinal fluid or BC samples matching the following criteria: (a) positivity within a 72-h incubation time between 1630 and 0800 on weekdays or 1200 and 0800 on weekends and (b) no prior positive identification of a microorganism in the same patient within the last 7 days, thereby eliminating follow-up BC samples with identical microbes. Before MALDI-TOF MS identification, Gram staining was carried out immediately following the positive readout of the BC incubator (Fig. 2A). If no bacteria were detected by Gram staining, the positive readouts of the BC incubator were classified as false positives. If bacteria were detected, the bacteria were directly identified from BC fluids by MALDI-TOF MS. Specifically, bacteria were extracted from 4–6 mL BC fluid using serum gel Monovette® tubes (Sarstedt). Monovette tubes were centrifuged at 3000g to separate cellular components from the BC fluid. The BC fluid supernatants were discarded and bacteria on top of the gel layer were suspended in 1 mL of water. Bacteria were washed once in water and twice in ethanol (75%), dried, and solved in a solution of 50% acetonitrile in formic acid. For bacterial identification by MALDI-TOF MS, we used an Autoflex II MALDI-TOF MS mass spectrometer (Bruker Daltonics) equipped with flexControl 3.0 and BioTyper 2.0 software running the database V3.1.2.0_3740–3995. Biotyper results with a score of ≥1.9 were validated and reported at the species level and archived in the laboratory information system.

### Fig. 2. Working steps for routine Gram staining (A) and routine MALDI TOF MS analysis (B) from the beginning of analysis to reporting.

Calculated TATs (C) for bacterial identification from first-encounter blood cultures by 4 different workflow concepts including MALDI-TOF MS or biochemical analysis (e.g. by a VITEK instrument) under idealized continuous or real discontinuous conditions in box-and-whisker plots. The horizontal lines within the boxes indicate the median. The boxes denote the interquartile range and the whiskers the 2nd or 98th percentile, respectively. The dots represent the outliers. LIS, laboratory information system.
log files of the BC incubator and the times required for the MALDI-TOF MS analyses as determined by a detailed work flow analysis.

The TATs for the control identification regimens were determined as the total time elapsed from reception of the BC to bacterial identification using a VITEK instrument (bioMérieux). This time included all between handling and incubation steps being carried out discontinuously during regular duty hours only or continuously under ideal conditions. To calculate the TAT for the control identification regimens, preincubation and postincubation lag times were added to the TTP of samples that either arrived at the laboratory or turned positive outside the regular duty hours. For discontinuous biochemical identification (Fig. 1), including Gram staining, subcultivation, and biochemical identification, we assumed a referenced mean TAT of 48 h. For continuous biochemical BC processing (Fig. 1), we subtracted a 6-h lag time twice for subculturing and identification, respectively, and assumed a mean TAT of 36 h.

**DATA COLLECTION**

Time stamps for registration times, Gram staining, and MALDI-TOF MS results were collected from the laboratory information system for 912 BC samples processed during a consecutive 140-day observation period. The anonymized relevant data were extracted from the database for further analysis. The time points for the start of BC incubation and the detection of a positive signal were collected from the BACTEC BC incubator log file.

**STATISTICAL ANALYSIS**

A paired t-test was performed to determine the statistical significance of the estimates for the TAT. Statistical analysis was performed using Microsoft Excel 2002 SP-2.

**Results**

Of 912 BCs, 549 (60%) turned positive outside the regular duty hours, showing an expected circadian distribution. Of these 549 positive samples, 207 (38%) fulfilled the criteria of first encounter and positivity within 72 h and thus were subjected to Gram staining immediately. In case of microbe detection, the BCs were further analyzed by MALDI-TOF MS. The mean TTP was 17.6 h (Table 1). Outside regular working hours the clinical chemistry emergency laboratory was run with reduced technical staff and responded to a considerable analytical workload in clinical chemistry, hematology, hemostaseology, and toxicology. Taking into account this high degree of capacity utilization, we implemented a batch-mode procedure for MALDI-TOF MS analysis outside regular working hours. During these laboratory hours, we measured a mean TAT from BC reception until the bacterial identification MALDI-TOF MS report of 24.9 h (Fig. 2C), represent-

### Table 1. Mean processing, lag, incubation, TATs, and TAT differences (in hours) of positive first-encounter BCs that fulfill criteria as described in Material and Methods.

<table>
<thead>
<tr>
<th></th>
<th>(I) Idealized projection continuous MALDI ID</th>
<th>(II) Batch-wise MALDI ID</th>
<th>(III) Idealized projection continuous VITEK ID</th>
<th>(IV) Regular duty VITEK ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preincubation delay</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>5.9</td>
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<tr>
<td>TTP</td>
<td>17.6</td>
<td>17.6</td>
<td>17.6</td>
<td>17.6</td>
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<tr>
<td>Gram stain (outside duty hours)</td>
<td>1.1</td>
<td>1.6</td>
<td>1.1</td>
<td>—</td>
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<tr>
<td>Postpositivity delay</td>
<td>—</td>
<td>6</td>
<td>0.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Gram stain (duty hours)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.1</td>
</tr>
<tr>
<td>Identification</td>
<td>1.2</td>
<td>1.2</td>
<td>36</td>
<td>48</td>
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<td>TAT Gram stain only</td>
<td>18.8</td>
<td>19.3</td>
<td>18.8</td>
<td>31.8</td>
</tr>
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<td>TAT Gram stain + bacterial ID</td>
<td>20</td>
<td>24.9</td>
<td>53.9</td>
<td>78.7</td>
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<tr>
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<td>—24.8</td>
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</tbody>
</table>

* Analyzed by 4 different work flow concepts. Work flow I was a sequential one for which the Gram-staining result was a primary criterion for MALDI-TOF ID. In scenarios II through IV, the Gram stains were performed during lag or incubation phases and did not influence the overall TAT for bacterial identification.
ing a processing and lag time of 7.2 h beyond the mean TTP of 17.6 h.

With reduced staff availability during night shifts, the mean TAT of the Gram-staining procedure measured from TTP to the Gram-staining report was 1.6 h (Table 1, work scenario II). Because the Gram stainings done under work scenario II conditions were carried out within the postpositivity lag phase before definitive bacterial identification, their processing times did not influence the overall TAT. In contrast, the Gram-staining procedure was measured at 1.1 h during working hours with unrestricted staff time. The Gram-staining processing time comprised (a) checking of sample identity and criteria for immediate processing, (b) BC retrieval and sampling under sterile conditions, (c) technically performing the Gram-staining protocol, (d) microscopic evaluation, (e) internal documentation, (f) telephone report to the ward, and (g) final documentation of the Gram-staining result in the laboratory information system. This process resulted in a mean TAT of 18.8 h from BC reception to the Gram-staining report (Fig. 2A, Table 1).

In batch mode, the bacterial identification by MALDI-TOF MS took approximately 1.2 h for the first and approximately 10 min for each additional sample from harvesting the bacteria to the final report (Fig. 2B). In addition, we allowed general reaction and handling times of approximately 10 min for each sample. In contrast, for the continuous uninterrupted work flow conditions for single-sample analysis, the complete handling and lag time of a positive BC, including Gram staining and subsequent bacterial identification, could be reduced to 2.4 h after TTP. Thus, the overall TAT from reception of the BC, including preincubation delay, to positivity (17.7 h) and the subsequent steps, including Gram stain and MALDI-TOF MS report (2.3 h), amounted to 20 h under the model conditions (Fig. 2C; Table 1, work scenario I).

To compare the TAT of bacterial identification by MALDI-TOF MS with the classical biochemical identification in the clinical microbiology laboratory, 2 different work flow scenarios were envisaged, a continuous protocol operable in a 24/7 laboratory and a discontinuous work flow performing BC reception, incubation, processing, and biochemical identification only during regular duty hours (in our institution, 08:00–16:30 h on weekdays and 08:00–12:00 h on weekends and holidays) (Fig. 1).

Of 912 evaluated BC samples, 392 samples (43%) both arrived in the laboratory and turned positive outside of the regular duty hours. Additionally, 447 samples (49%) arrived in the laboratory inside regular duty hours and the results turned positive outside of these hours or vice versa.

For BC received in a routine microbiology laboratory outside the regular duty periods, there was a mean preincubation lag time of 5.9 h. Furthermore, BC turning positive outside the regular duty periods had a mean postincubation lag time of 7.2 h (Table 1). Accordingly, the total TAT of the whole procedure until the Gram-staining report took a mean of 32 h. These lag times would not exist in a 24/7 clinical microbiology laboratory with continuous BC reception and processing (Fig. 1, Table 1). The classical procedure of cultivation on solid selective media with subsequent biochemical identification, e.g., by a VITEK II instrument, requires approximately 48 h with handling performed during regular duty hours only. Accordingly, the total TAT from BC reception to the bacterial identification report was a mean of 78.7 h (Fig. 2C, Table 1). An optimized work flow for biochemical bacterial identification in a 24/7 microbiological laboratory would include continuous BC incubation and processing, Gram staining, clonal cultivation on selective agar plates, and biochemical identification of bacteria. In this scenario, the lag phases occurring before VITEK loading as well as data validation and release would be avoided, resulting in an improved TAT between BC reception and final report of 53.9 h altogether (Fig. 2C, Table 1). Because the Gram stainings were performed during growth phases on solid media in both work flow scenarios, the processing times were not added to the overall TAT for bacterial identification.

**Discussion**

It is considered axiomatic that fast TATs improve management of patients with septicemia. Accordingly, the employment of new technologies aims to advance patient outcomes while decreasing healthcare costs and nosocomial infections with multiresistant bacteria (13, 14, 15, 17). Very recent reports have shown inconsistent results of the clinical impact of faster microbial testing using PCR and MALDI-TOF on hospital stay and cost reductions (8, 17).

Direct identification of bacteria from BC through the use of MALDI-TOF MS comprises a complex work flow that needs to be available in a 24/7 fashion to be most effective. Also important is how much lead time is gained with an immediate response to a BC microbial growth alarm compared to a batch-wise analysis of positive BCs. This is particularly important for clinical diagnostic laboratories that also provide service in the area of clinical chemistry, hematology, and hemostaseology.

In this study we therefore investigated 4 work flow models of microbiological testing governed by regular duty hours or a 24/7 availability. Because approximately two-thirds of BC samples are received outside
regular laboratory hours, our aim was to identify the greatest timesavers in the respective realistic clinical laboratory setting, i.e., under the constraints of limited laboratory staff.

In 92% of cases, either the arrival of BC samples in the laboratory or their positivity fell outside of the regular duty hours. Therefore, 24/7 continuous BC incubation with further processing would accelerate microbiological diagnostics by several hours compared to a discontinuous work flow. In our hands, the mean TATs for Gram staining and for biochemical bacterial identification could be reduced by approximately 12 h and in some cases up to 30 h by changing only the BC reception/incubation procedure from a discontinuous into a continuous 24/7 process.

Furthermore, compared to processing BC in regular duty hours only, a 24/7 availability of biochemical characterization by a microbiological service reduced the mean TATs for biochemical identification by an additional 12 h. This was primarily due to the fact that subculturing and identification steps were being set up during regular duty times but produced results that statistically fell into the off-duty hours and would not be processed until the next day. Taken together, these changes reduced the mean TAT by approximately 25 h.

We further investigated the effects of implementing bacterial identification by immediate or batch-wise MALDI-TOF MS analysis into the 24/7 BC reception/incubation. Direct MALDI-TOF MS analysis from BCs has been shown to be successful in 72% to >95% of these situations (13, 15, 18). In the case of some streptococci that are notoriously difficult to differentiate by MALDI-TOF MS, the combination with an immunochromatographic test has resulted in a further improvement of diagnostic performance (13).

The time we measured for bacterial identification using MALDI-TOF MS is in accordance with that of other model reports. In contrast, we investigated bacterial identification by MALDI-TOF MS under real laboratory conditions during night shifts with reduced routine staff availability and a full workload of other clinical chemistry and microbiological diagnostic tasks.

As expected, the introduction of continuous BC incubation and batch-wise MALDI-TOF MS analysis with a discontinuous BC incubation and biochemical bacterial identification during regular duty times resulted in a reduction in TAT by 53.8 h. This TAT reduction could be further enhanced by 4.9 h, to a 58.7-h TAT, by increasing the staff availability in the 24/7 laboratory, thus allowing for a more immediate MALDI-TOF MS response. Under 24/7 BC reception/incubation, the comparison of a continuous biochemical identification with the immediate response MALDI-TOF MS analysis would yield an advantage in TAT of 33.9 h.

Other studies confirm our TAT calculations for discontinuous BC incubation and biochemical bacterial identification and have demonstrated TAT reductions between 73 and 83 h for the bacterial identification report with the use of MALDI-TOF MS analysis (18, 19). These results suggest that our work flow analysis has correctly identified the work flow components critical for improvement of this important diagnostic procedure. It further seems reasonable to assume that mean assay time and laboratory organization, in addition to the time to positivity, are the major determinants of the mean TAT, because work flows in today’s clinical laboratories are highly standardized and reproducible, and thus make the mean time required for a particular process highly predictable.

The reduction in TAT of 58.7 h with the use of an optimized immediate response MALDI TOF MS regimen seems to be a reasonable assessment. Further studies would be required to investigate whether immediate response or batch-mode MALDI-TOF analysis would make a difference for patient outcomes. Perez et al. (17) have shown convincingly that new laboratory procedures need active stewardship to be effectively accepted in a clinical setting dealing with critically ill patients.

Nevertheless, our study shows that TATs for biochemical identification methods would also profit from continuous BC incubation and processing. For the theoretical scenario of continuous BC incubation, Gram staining, plating, and discontinuous bacterial identification, we calculated a TAT reduction of 12.8 h compared to discontinuous BC incubation and processing. However, even a reported optimized biochemical identification protocol (20) still does not meet the TAT for the batch-wise or immediate MALDI-TOF MS identification, as demonstrated in this study. Thus, our data suggest that MALDI-TOF MS in combination with continuous sample processing is one of the most rapid methods for bacterial identification in a clinical setting.

The benefits of timely microbiological testing of blood cultures for patient management are under active investigation and may arise primarily from optimized and pathogen-directed antibiotic use (16, 20, 21), resulting in decreases in side effects, antibiotic resistance (22, 23), and therapy costs (16, 17). The first published evidence suggests that faster bacterial identification by MALDI-TOF MS or PCR does indeed increase the appropriateness of knowledge-based antibiotic treatment of bacteremia (24, 25), properly reducing therapy and hospitalization costs (17). As observed by Frye et al. (6), the actual cost savings by accelerated bacterial
TOF MS has the potential to enable testing for antimicrobial therapy. However, in addition to pathogen identification, microbiological testing usually also includes information about susceptibility testing, which MALDI-TOF MS is not yet established to deliver. Evidence that MALDI-TOF MS has the potential to enable testing for antimicrobial susceptibility testing has been published (26–29), and thus future research might unveil the potential of MALDI-TOF MS to incorporate susceptibility testing.

In conclusion, we have demonstrated that immediate processing of blood cultures by a laboratory operating 24/7 in combination with MALDI-TOF MS identification substantially reduces the time required for bacterial identification in a clinical setting. Further studies will have to investigate whether rapid bacterial identification by MALDI-TOF MS has an impact on clinical decision-making and consequently is effective in improving the outcome of patients with acute infectious diseases.

References


