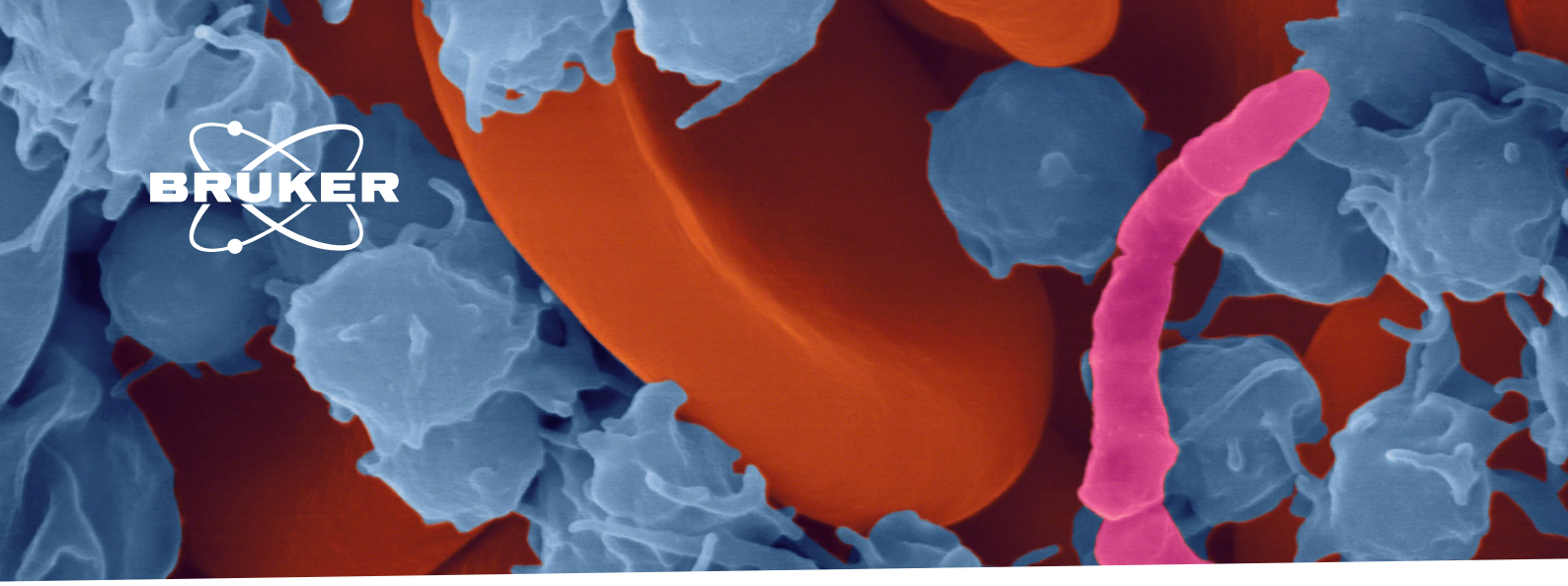

Comparison of different short culturing methods to the Rapid Sepsityper workflow: Microorganism identification from positive blood cultures

Authors: Miriam Cordovana, Hilke Hubert, Markus Meyer, Arthur B. Pranada, Jörg Steinmann

Introduction

Sepsis is still one of the leading causes of death worldwide, burdened by high morbidity and mortality rates (20–50%) [1]. Since the survival rate of not properly treated patients decreases by the hour [2], rapid identification (ID) of the causative microorganism and antimicrobial susceptibility testing (AST) are crucial for the patients' clinical outcome [3–5]. Blood cultures (BCs) are the gold standard for diagnosis of bloodstream infections [5], but traditional approaches for the identification of microorganisms from BCs are slow. MALDI-TOF MS technology has been widely investigated to speed up the time-to-report for ID from positive blood cultures, to deliver a "same-day result". Many different procedures and protocols were developed, all of them attributable to two main approaches: the short subculture, which is based on plate culturing and incubation for a short time (2–6 h), and the direct enrichment and separation of microbial cells from the positive blood culture bottle [6]. The short subculture methods do not require extensive sample handling (or require just a minimal one), but they are slower than direct enrichment approaches, and are not suitable for slow-growing and fastidious species. The direct methods require a sample pre-treatment to purify the microorganisms from all non-microbial material present in the blood (that would interfere with the MALDI-based ID method), but are still fast (less than 1 h), and enable the ID of virtually every species included in the MALDI Biotyper® library.

The MBT Sepsityper® IVD Kit (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) is the only IVD-CE labelled kit which allows the microorganism ID directly from a positive BC by MALDI-TOF MS within 15–20 min [7]. The Rapid Sepsityper workflow (commercially available since 2018) includes a blood cell lysis step (not disruptive for microorganisms), followed by centrifugation and washing steps, to obtain a pure microbial pellet. This pellet, a biomass of living microorganisms, can be used for ID and for further downstream applications, significantly shortening the handling and the reporting time.

In this study, the Rapid Sepsityper workflow – using the MBT Sepsityper IVD Kit – was compared to three different short subculturing methods. Routine samples from three clinical microbiology laboratories (Institut für Klinikhygiene, Medizinische Mikrobiologie und Klinische Infektiologie Klinikum Nürnberg; Microbiology Unit of the University Hospital of Bologna

IVD

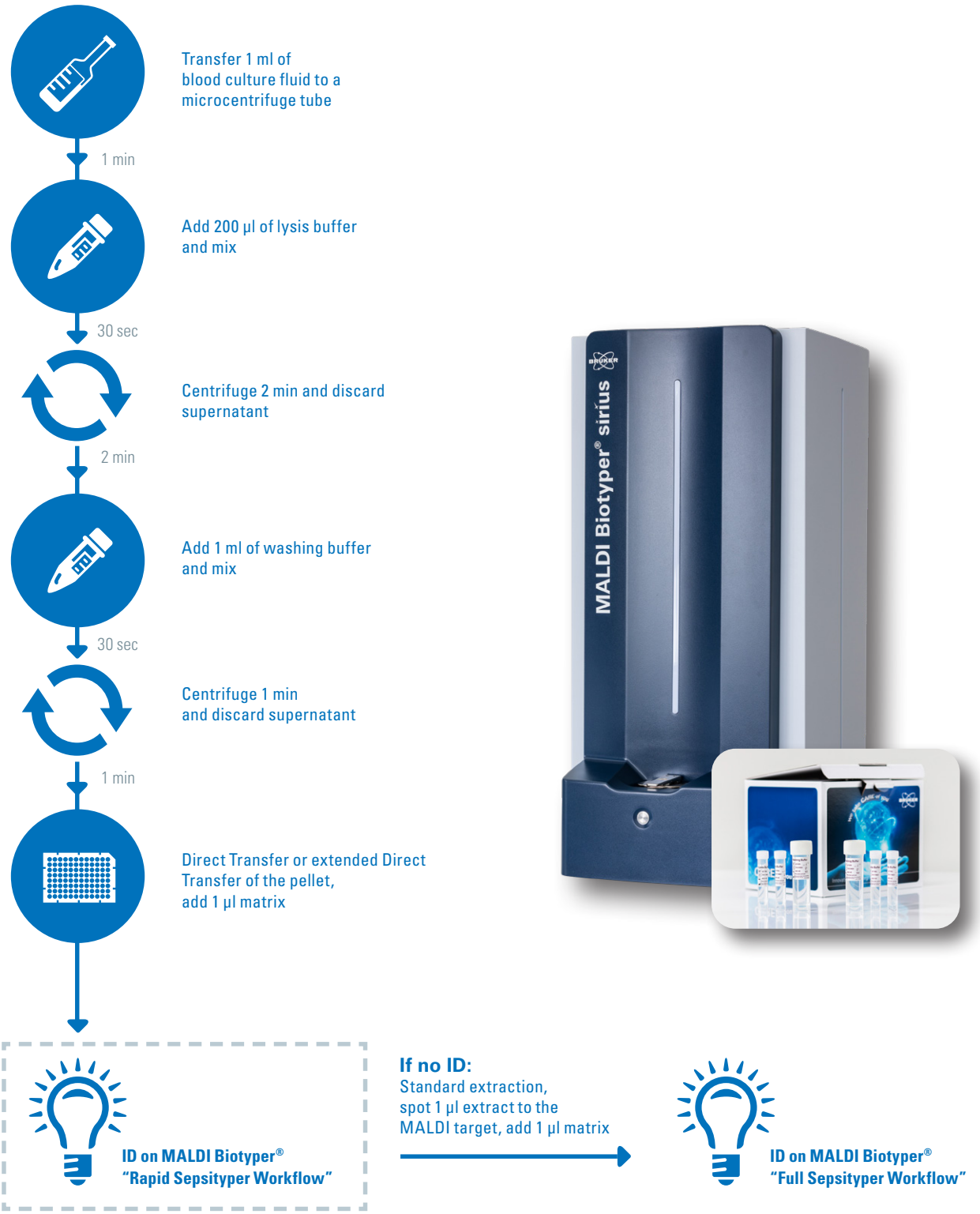

Figure 1
Rapid workflow

Figure 2
Comparison of Rapid Sepsityper results with those of short subculture for gram negatives, gram positives, anaerobes and yeasts (% of correct ID)

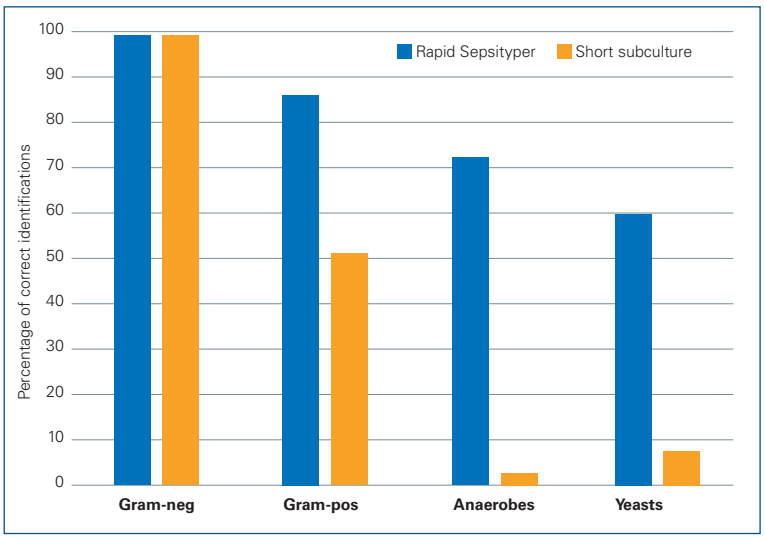

Table 1
Media and conditions used for the short subculturing methods

Laboratory	Agar	Method	Volume of PBC used	Temp.	Time
Dortmund	Columbia Blood Agar	Direct plating	20 µl	35 ±2°C	2.5 h and 5 h
Nuremberg	Columbia Blood Agar	Dilution: 500–600 µl in 1 ml of 0.45% NaCl solution	100 µl of diluted sample	35 ±2°C	4 h
Bologna	Chocolate Blood Agar	Enrichment by centrifugation with gel separator	8 ml	35 ±2°C	1.5–2 h

Policlinico Sant'Orsola-Malpighi; and MVZ Dr. Eberhard & Partner Dortmund, Department of Medical Microbiology, Dortmund, Germany) were used to compare the Rapid Sepsityper workflow to their respective established short subculturing method. Details of the short subculturing methods are shown in Table 1.

Methods

A total of 498 routine patient-derived samples were analyzed in this study, including aerobic, anaerobic, and pediatric bottles (BACTEC™ Plus Aerobic/F, BACTEC™ Plus-Lytic/10, BACTEC™ Peds Plus™/F, Becton, Dickinson and Company, Sparks, MD, USA), processed using the BD BACTEC™ FX system (Becton Dickinson). All samples underwent in parallel Gram staining, rapid ID (by short subculture and Rapid Sepsityper) and standard culture.

Rapid Sepsityper

The Rapid Sepsityper sample preparation was performed following the Instructions for Use, workflow see fig. 1. Briefly, 1 ml of the blood culture sample was transferred to a 1.5 ml tube. After adding 200 µl lysis buffer, the samples were immediately mixed by inversion, vortexed for 5–10 sec, and centrifuged for 2 min at 15,000 rpm. The supernatant was discarded, and the pellet was resuspended in 1 ml of washing buffer and centrifuged for 1–2 min at 15,000 rpm. The supernatant was removed, paying attention to eliminate any residual liquid. The pellet was directly smeared in duplicate onto the MALDI Biotyper target using a 1–10 µl pipette tip. One spot was covered by 1 µl of 70% formic acid and air-dried, before the addition of 1 µl of HCCA matrix. The other spot was air-dried and 1 µl of HCCA matrix was applied directly – Fig. 1.

	Dortmund				Nuremberg			Bologna		
Microbial groups	**number of samples n**	**ID Sepsityper n (%)**	**ID short subculture 2.5 h n (%)**	**ID short subculture 5 h n (%)**	**number of samples n**	**ID Sepsityper n (%)**	**ID short subculture 6 h n (%)**	**number of samples n**	**ID Sepsityper n (%)**	**ID short subculture (choc.) 2 h n (%)**
Enterobacterales	57	57 (100)	52 (91.2)	57 (100)	24	23 (95.8)	12/23 (52.2)*	48	48 (100)	48 (100)
Non-fermenting gram negatives	3	3 (100)	1 (33.3)	3 (100)	3	3 (100)	2 (66.7)	13	12 (92.3)	12 (92.3)
S. aureus	29	29 (100)	17 (58.6)	23 (79.3)	6	6 (100)	2/4 (50)*	11	11 (100)	10 (90.9)
CoNS	61	54 (88.5)	12 (22.2)	30 (55.6)	37	31 (83.8)	22/36 (61.1)*	20	20 (100)	11 (55.0)
Enterococci	13	13 (100)	3 (23.1)	9 (69.2)	2	2 (100)	1 (50)	8	8 (100)	8 (100)
β-hemolytic streptococci	1	0	0	1 (100)	2	2 (100)	0	2	2 (100)	2 (100)
S. pneumoniae	2	0	0	0	4	0	4 (100)	5	1 (20)	1 (20)
Viridans streptococci	4	4 (100)	0	0	10	9 (90.0)	4/8 (50)*	4	3 (75)	2 (50)
Gram+ rods	3	2 (66.7)	0	0	5	2 (40.0)	1 (20)	6	3 (50)	1 (16.7)
Anaerobes	2	0	0	0	15	12 (80.0)	1/14 (7.1)**	13	10 (76.9)	0
Yeasts	3	2 (66.7)	0	0	13	7 (53.8)	2/12 (16.7)*	14	9 (64.3)	0
Total mono-microbial	**178**	**164 (92.1)**	**92 (51.7)**	**123 (69.1)**	**121**	**97 (80.2)**	**50 (44.2)**	**144**	**128 (88.9)**	**95 (66.0)**
Total poly-microbial	**21**	**18 (85.7)**	**7 (33.3)**	**9 (42.8)**	**19**	**17 (89.5)**	**8 (42.1)**	**15**	**13 (67.7)**	**8 (53.3)**

Table 2
Comparison of the results of the Rapid Sepsityper workflow with those of short cultivation at three different study sites (in total 498 samples)
* 8 samples in total (out of all bacterial groups) were not analyzed following the short subculture method
** *Lactobacillus paracasei*

Short subculture

The subculturing methods were performed using Columbia sheep blood agar, one including the subculture of 100 µl of diluted sample for 6 h (Nuremberg), the other including the subculture of 20 µl of sample for 2.5 and 5 h (Dortmund), both at 35°C in an aerobic environment. The third subculturing method (Bologna) started with an enrichment of bacterial cells by centrifugation of 8 ml of blood culture in a tube with a gel separator (Vacutainer SST tubes, from BD) for 10 min at 3,000 rpm. The supernatant was discarded, and the pellet, resuspended in 50 µl of NaCl (0.9%), was then streaked onto a chocolate blood agar plate, and incubated for 1.5–2 h at 35 ±2°C in an aerobic environment.

The colonies/bacteria from confluent growth were spotted onto a MALDI Biotyper target and 1 µl of HCCA matrix was added (DT and eDT method). All details of the short culturing methods are summarized in table 2.

	No ID Sepsityper (n, %)	No ID short subculture (n,%)
Enterobacteria	1 (1.8)	11 (6.7)
Non-fermenting Gram negatives	1 (1.8)	2 (1.2)
S. aureus	-	9 (5.4)
CoNS	13 (23.6)	54 (32.5)
Enterococci	-	5 (3.0)
Beta-haemolytic strepto-cocci	1 (1.8)	2 (1.2)
S. pneumoniae	10 (18.2)	6 (3.6)
Viridans streptococci	2 (3.6)	10 (6.0)
Gram-positive rods	7 (12.7)	12 (7.2)
Anaerobes	8 (14.5)	28 (16.9)
Yeasts	12 (21.8)	27 (16.3)
Total NO ID	55	166

Table 3
Summary of non-identified samples

Rate of identification
The ID results delivered by both methods – Rapid Sepsityper and short subculturing – were compared with results provided by the respective overnight subculture and with Gram staining results.
The rate of correct ID was calculated for the main microbial "groups" (*Enterobacterales*, non-fermenting gram-negative bacilli, *S. aureus*, coagulase-negative staphylococci, enterococci, streptococci, gram-positive aerobic bacilli, anaerobes, and yeasts).

Results

Overall, of the 498 samples 443 were monomicrobial, 50 were polymicrobial, 5 were false positives. Among the monomicrobial samples, 148 aerobic gram-negative bacilli (33.4%), 235 aerobic gram positives (53.0%), 30 yeasts (6.8%) and 30 anaerobes (6.8%) were grown.
A summary of all results of the Rapid Sepsityper workflow and short subculture methods for the three study sites is shown in Table 2.

Rate of correct ID with Rapid Sepsityper
All in all, the Rapid Sepsityper method enabled the ID of 388/443 (87.6%) monomicrobial samples and of 48/55 (87.3%) polymicrobial ones (in all cases, one of the two species was identified). The failed identifications involved mainly CoNS, yeasts and *S. pneumoniae* (see Table 3).

Rate of correct ID with short subculture
In total, merging results of the three methods, short subculture enabled the ID of 271/443 (61.2%) monomicrobial samples, and of 25/55 (45.4%) polymicrobial ones (one of the two species identified). Failed identifications involved mostly gram-positive species in general, yeasts and especially anaerobes (intrinsic limit of the methodology) (Table 3).

Summary and Conclusion

In this study, the MBT Sepsityper® IVD Kit with its Rapid workflow proved to be superior to three different short subculturing methods, which represented the routine standard of three different clinical microbiology laboratories. In addition to its significantly shorter time-to-report, the Rapid Sepsityper workflow resulted in a higher rate of correct identifications, both in terms of total ID and for each different microbial group.

The two approaches showed a similar and excellent performance for *Enterobacterales*, non-fermenting gram-negative bacilli, *S. aureus* and *enterococci*. On the contrary, for the slower growing clinically relevant species/families, the Rapid Sepsityper workflow showed a far better performance, especially for yeast, CoNS, gram-positive bacilli, and streptococci. Furthermore, as short subculture methods rely on incubation in aerobic environment, the anaerobes cannot be identified with such methods, while the Rapid Sepsityper workflow rendered a good identification rate (see Fig. 2).

In case of samples identified by both methods, the Rapid Sepsityper workflow delivered an actionable result 3–4 h earlier than short subculture, while for yeast and anaerobes, the time-to-report was dramatically shortened by 24–48 h.

A more detailed insight into and interpretation of the conducted comparison can be found in [8].

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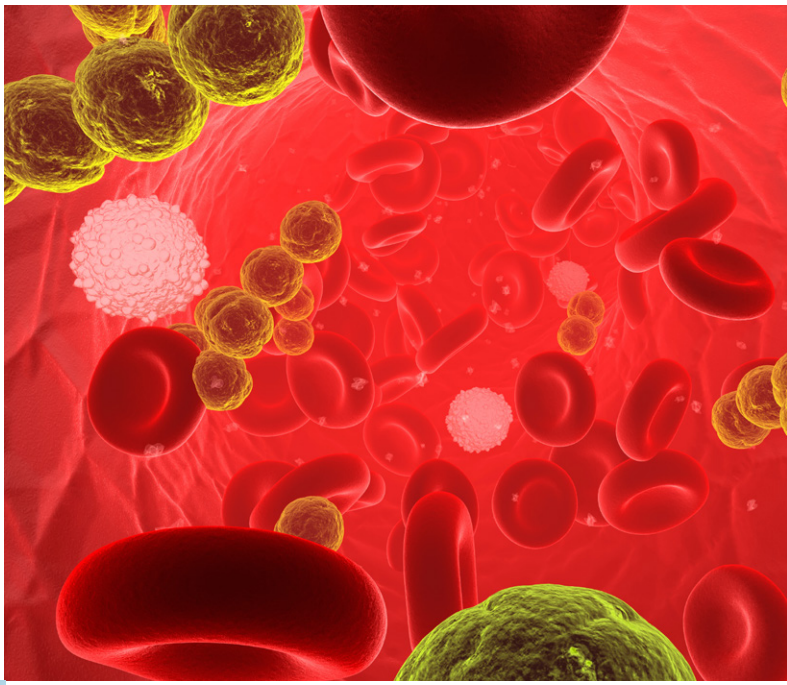

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