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► **To cite this version:**

Brigitte Lamy, Martin Sundqvist, Evgeny A. Idelevich. Bloodstream infections – Standard and progress in pathogen diagnostics. *Clinical Microbiology and Infection*, 2020, 26, pp.142 - 150. 10.1016/j.cmi.2019.11.017 . hal-03489942

HAL Id: hal-03489942

<https://hal.science/hal-03489942v1>

Submitted on 7 Mar 2022

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1 Invited review article for THEME ISSUE on BLOODSTREAM INFECTION

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4 **Bloodstream infections – standard and progress in pathogen diagnostics**

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23 **Key words:** blood culture, rapid diagnostics, bloodstream infection, logistics, MALDI-TOF

24 mass spectrometry, bundle approach, rapid antimicrobial susceptibility testing, rapid

25 identification

26 **Abstract**

27 **Background:** Bloodstream infection (BSI) is a major public health burden worldwide, with
28 high mortality. Patient outcome is critically influenced by delayed therapy, and fast and
29 accurate pathogen diagnostics decisively improves the care of patients. During the past two
30 decades major improvements has been made in the diagnostic performance of blood culture
31 diagnostics through actions on pre-analysis and time-to-result.

32 **Objectives:** To review and discuss the literature for standard procedures and the progress in
33 BSI pathogen diagnostics, and to propose a new mindset to reach an improved diagnostic
34 workflow.

35 **Sources:** Scientific articles and reviews available through NCBI/Pubmed.

36 **Content:** Blood culture performance relies largely on the quality of its pre-analytical phase
37 that is improved with educational actions monitored by using key performance indicators, and
38 external quality assessment. Advanced blood culture systems now provide tools for an
39 automated estimation of the bottle filling. These proved efficient to facilitate effective training
40 for improving blood collection. On analytic aspects, rapid methods for pathogen
41 identification, among which matrix-assisted laser desorption/ionization time of flight mass
42 spectrometry dominates, and rapid antimicrobial susceptibility testing are reviewed. These
43 technical developments call for improvements in all other steps, especially in pre- and post-
44 analytic logistics to give the full reciprocation of these techniques on patient management.
45 This aspect is summarized in the term of “microbiologistics” that covers all possible
46 improvements in the logistic chain from sampling to report.

47 **Implications:** Progress in BSI pathogen diagnostics is based on a bundle approach that
48 includes optimization of the pre-analytical parameters, rapid start of incubation, the use of
49 rapid methods, re-organisation (e.g. 24/7, transportation service) and a close involvement of
50 antimicrobial stewardship teams. These developments lead to define a new standard for
51 bloodstream infection diagnostics.

52 **Introduction**

53 With an estimated burden of 1,200,000 episodes of bloodstream infection (BSI) each year in
54 Europe and long-term sequelae, BSI represents an increasing public health concern [1].
55 Delayed effective therapy is associated with worse outcome [2,3]. Fast and accurate diagnosis
56 of the causing microorganism and a correct susceptibility testing improves the care of patients
57 with BSI/sepsis and leads to a more precise therapy which grows in importance with
58 antimicrobial resistance increase [4-7]. The detection of the causing pathogen with molecular
59 techniques has, so far, been proven suboptimal [7] and blood culture (BC) remains the gold
60 standard and first line tool in the pathogen diagnostics of BSIs and sepsis [7].

61 The introduction of continuous monitoring blood culture systems (CMBCS) in the 1990s led
62 to an improved standardization of BC diagnostics with easier ordering and optimized culture
63 media yield [8]. However, this development was not followed by other improvements in the
64 area for the next decades while CMBCSs partly obscured the fact that pre-analytics are
65 critical to detect BSI pathogen. During the last decade, however, a dramatic progress in the
66 development of rapid diagnostic tests relying on innovative technologies has occurred [9].
67 Also, the importance of the logistics and the improvement of quality management of BC
68 diagnostics are increasingly recognized [10]. This resulted in a multifaceted range of actions
69 to improve the microbiological diagnosis of BSI (Fig.1), and many of them are
70 complementary. Here, we review the standards of BSI pathogen diagnostics, the progress in
71 this area, in terms of reduced time to result (TTR) and quality of results and propose new
72 timelines to aim for in modern BC diagnostics.

73

74 **Standards in BSI pathogen diagnostics**

75 The performance of BSI diagnostics is to a large extent determined by the pre-analytical
76 process including the skin preparation, the amount of blood per bottle, the number of bottles

77 collected per episode and the time to bottle incubation [11,12] (Table 1). It is however
78 striking how far real-life conditions in many cases are from recommendations [11,13-15].
79 The classical analytical process of microbiological BSI diagnostics that prevailed until end of
80 2000s is shown in Fig. 2. However, the introduction of new methods in clinical microbiology
81 deeply rushed practices during the latest decade and outdates the classical process. The high
82 variability in utility, dissemination and cost of the new techniques (like matrix-assisted laser
83 desorption/ionization time of flight mass spectrometry (MALDI-TOF MS)) makes it
84 challenging to define the current standard of BSI pathogen diagnostics [13]. In the absence of
85 dedicated guidelines the novel methods have been adopted very differently [13]. Before the
86 introduction of MALDI-TOF MS, the typical TTR was 2-4 day for a positive BC, which is
87 too slow for affecting acute patient management. However, in European microbiology
88 laboratories, MALDI-TOF MS systems have now almost completely replaced time-
89 consuming biochemical identification [13]. The full process of BC diagnostics are thus able to
90 move towards the lowest possible TTR and at the very latest a final species identification and
91 an AST report should be available within 24h following bottle positive signal (Table 1). To
92 reach this target and also increase the sensitivity and specificity of the BC process, several
93 steps need to be addressed.

94

95 **Progress in sampling procedures, blood volumes and quality management**

96 An increasing awareness of the importance of pre-analytics in BSI diagnostic quality is seen
97 through a number of initiatives for improvement, the recognition of the low achievement of
98 the recommended targets for contamination and blood volume, and not least the recognition
99 of the need for extra tools to visualize and promote improvements [10,16].

100 Several approaches are available to address the quality challenges in BSI pathogen
101 diagnostics. First, continuous quality improvements have grown in importance [10]. They
102 include, in addition to quality management systems, implementation of relevant key

103 performance indicators (KPI) to monitor critical points in the BC process that mostly focus on
104 critical pre-analytical criteria [10] (Table 2). Such monitoring easily highlights what pre-
105 analytical factor needs to be improved, and training (e.g. of nurse or phlebotomists) has thus
106 become pivotal to improve e.g., bottle filling and the quality of skin preparation [14,15].
107 However, adequate filling may be moderate also in post-intervention assessments [15] and
108 training effects may also fade with time, which calls for new strategies to reach effective
109 training. Second, manufacturers have implemented technical features for automated
110 estimation of the bottle filling in CMBCSs either with a direct or an indirect estimation of the
111 amount of blood cultured (Table 2) [17,18]. Data are easily acquired and can be used as an
112 educational tool for quality improvement on the departmental level as summarized in Table 2
113 [14,15]. Third, initiatives to promote external quality assessment (EQA) of the BC pre-
114 analytical quality in European hospitals have been instituted (www.ctcb.com). The current
115 level of quality at a population level (laboratories) is measured, and the EQA helps
116 monitoring the efficiency and actions of improvement at a hospital/community level [19,20].
117 Although none of these approaches directly improves the pre-analytical quality per se, the
118 specific steps in the process are better visualized and targets can be set to improve the BC
119 quality.

120 Promising strategies to prevent contamination include, as recently reviewed [12], the single
121 puncture sampling [11] and the diversion technique as presented in Table 2. The latter
122 consists in diverting the first ml blood either in an additional sample tube or using a dedicated
123 device, which reduces contamination to a varying extent in clinical studies [21,22].

124

125 ***Rapid identification from positive BC***

126 As MALDI-TOF MS is used in most laboratories across Europe for the species identification
127 from cultured isolates [13], it is reasonable to use this technology for further applications,
128 notably for shortening time to BSI pathogen diagnostics. Two major MALDI-TOF MS-based

129 approaches are available to reach rapid identification from positive BC: *direct identification*
130 *and identification using short sub-culture on solid medium* (Fig. 3).

131 The microorganisms in BC broth are not readily available for identification, and separation of
132 microorganisms is therefore required. In the direct identification, the BC suspension is usually
133 treated with detergent agent followed by a centrifugation procedure and in some protocols
134 protein extraction with ethanol and formic acid [23,24]. Finally a MALDI-TOF MS
135 measurement is performed [23-25]. This method enables very rapid identification (20-40 min
136 processing time), a report at the same time as the report of the Gram-stain and an impact on
137 patients' management [9,26,27]. However, the method has some limitations where suboptimal
138 integration into the workflow might lead to batch processing [25,27] with reduced effects on
139 patient management. The identification rates with this method can reach 80% except for
140 yeasts [23-25,28]. The problems with logistic changes in the laboratory seem to be less
141 pronounced with the identification from positive BC using short sub-culture on solid medium,
142 which is currently the most commonly used approach [13]. The method allows reliable
143 identification by MALDI-TOF MS after a short incubation step (only a few hours) without
144 any additional processing [23,29]. Mixed cultures cannot be reliably recognized by any of the
145 two methods, thus still warranting Gram-stain directly from the BC and incubation of agar
146 plates overnight for control. However, this does not constitute a significant drawback as only
147 a minority of positive BCs include more than one species [23,29].

148 Several commercial systems based on multiplexed PCR or microarray have been marketed
149 during the last years to allow the identification of bacterial and fungal pathogens from
150 positive BC within one to four hours [30-32]. Although giving a reliable result for the
151 included pathogens, they all share the common drawbacks of being expensive, only process a
152 few samples simultaneously and have limited panels of pathogens [30-32]. As the MALDI-
153 TOF MS-based methods provide identification from positive BC in similar time for a larger
154 range of microorganisms, with much lower cost for laboratories and can be very well

155 combined with rapid AST [33-35], the advantages of the molecular systems for rapid species
156 identification are not clearly recognizable.

157

158 ***Rapid AST from positive BC***

159 Timely AST report is essential for the definitive choice of targeted antimicrobial treatment.
160 The current possibilities for AST acceleration have recently been reviewed [36]. Practical,
161 affordable and automated systems that would allow “laboratory-friendly” high-throughput
162 rapid testing from blood or from positive BCs are currently not available for routine
163 diagnostics. Due to the long testing times, AST is usually not finished in the same working
164 shift [37,38]. Taking into account limited working hours of microbiology laboratories [13],
165 the AST report will only be available on the next day in most settings [37]. Nevertheless, it is
166 possible, and should be advocated, to start AST on the same day instead of application of
167 overnight colonies. By doing so, result is usually available one day earlier. The same-day
168 inoculation can be performed from positive BC by using a microbial pellet after
169 centrifugation [28,34,35,39], by inoculation of sub-cultures from positive BCs shortly (a few
170 hours) incubated on agar plates [40] or directly from positive BC [41,42]. With direct
171 inoculation of bacterial pellets, accurate results were achieved for Gram-negative rods
172 [34,35], but poorer accuracy was reported for Gram-positive cocci [35,39] and yeasts [28].
173 Inoculation of short sub-cultures from solid medium results in more accurate AST results
174 [40,43,44], probably due to a better inoculum standardization [40], and can be combined with
175 MALDI-TOF MS identification from the same short cultures in a practical process [33]. A
176 procedure for direct application of positive BC broth onto agar plates [41] and the breakpoints
177 for early result reading of this method [42] have recently been issued by the European
178 Committee on Antimicrobial Susceptibility Testing (EUCAST) after testing the concept in
179 clinical laboratories across Europe [45,46]. The method seems promising but further
180 evaluation is needed to assess workflow integration, including the visual reading of inhibition

181 zones and the potential need for multiple plate examinations. MALDI-TOF MS-based
182 universal phenotypic AST is one of the promising technologies in the development as it can
183 efficiently be combined with MALDI-TOF MS identification [34]. Polymicrobial blood
184 samples and species with slow growth represent a limitation for direct AST whatever method
185 is used [36,47,48].

186 Some molecular systems for microbial identification from positive BCs and from
187 whole blood have included selected resistance gene markers in their multiplex panels [30-32].
188 This information has been shown beneficial in case of *mecA* detection [49], but is in many
189 areas of limited practical value. Importantly, for many resistance mechanisms negative result
190 does not exclude phenotypic resistance due to other resistance mechanisms than those
191 included in the test [33,36,50]. Thus, physicians often do not change antibiotic regimen in the
192 light of only molecular results, with a loss of interest for such rapid diagnostics [51].

193

194 **DNA-based identification from whole blood**

195 As cultural diagnostics inevitably requires time for incubation of blood specimens,
196 molecular systems have been suggested for direct detection and identification of pathogens
197 from whole blood [52,53]. A number of studies have evaluated these systems regarding
198 diagnostic accuracy [54], and some studies have investigated their clinical utility in
199 retrospective [55,56] or prospective but not randomized [57,58] designs. Two randomized
200 controlled trials [59,60] showed a more rapid communication of species identification to the
201 clinicians [60] and earlier targeted antimicrobial therapy [59] when multiplex PCR was used.
202 There was no significant effect on mortality [59,60]. In general, molecular diagnostics
203 currently cannot replace BC [9,11,61] (Fig. 3). Furthermore, the clinical utility of direct PCR
204 from whole blood can only be fully exploited if specimen is processed directly after sampling.
205 As with all rapid methods, the molecular approaches need a 24/7 laboratory with skilled staff

206 to maximize the positive effects [62], something which is not established in most of European
207 microbiology laboratories [13] but has been implemented in more laboratories in the USA.

208

209 **Microbiologistics - simple ways of speeding up BC processing**

210 The implementation of MALDI-TOF MS and molecular approaches for species identification
211 and rapid AST can lead to substantial decrease in the TTR. However, it is important that the
212 introduction of these novel rapid methods also are accompanied by effective logistics in the
213 pre- and post-analytic steps so that the total time from the patient with suspicion of BSI enters
214 the hospital to a report is reduced (Fig.2). For this process, we think that the term
215 “microbiologistics” can be introduced as it in a witty manner includes all aspects of logistics
216 in microbiology. The total time to report and the quality of BC can be improved by shortening
217 the time to start the incubation of BC vials [63-65]. These include 4 main types of actions, all
218 complementary. First, a microbiology laboratory open 24/7 will ideally lead to a reduced time
219 to: the introduction of BC vials, processing of positive samples and also a rapid report to the
220 clinician [65]. The 24/7 approach also needs to be supplemented with optimal pre-analytic
221 transport chains of the samples [65] as shown in Table 3. Second, a cost-effective step is the
222 placement of the CMBCS outside the laboratory [63]. This step will in itself lead to more
223 rapid Gram-stain/species reports for most samples and will not lead to false negative results
224 [63]. The efficiency of this approach depends on the transport time of the positively flagged
225 bottles, and a timely transport to the microbiological laboratory to ensure the effectivity of
226 this approach is paramount (Table 3). Optimal transport of bottles either before or after
227 incubation is compelling and it relies on a continuous transport system. The use of automated
228 system (e.g., pneumatic tubes) that is independent from rounds at set times is preferred.
229 Finally, early introduction will lead to a more rapid process [66] and will lead to a higher
230 recovery of pathogens [64,67].

231 After optimal transport and analysis it is important that the result is communicated rapidly
232 [68]. The report should be electronic and contain a very clear guidance regarding the
233 importance of the pathogen and AST result [69] or even better, additionally be communicated
234 with an antibiotic stewardship team [31,70] to reach full clinical value of the BC result.

235

236 **Critical appraisal of progress**

237 Despite many technical developments, BC is still the foundation in BSI pathogen diagnostics.
238 We have here shown many possibilities to reduce the TTR and outlined the possible new
239 standards to reach for BC diagnostics. Still, too many microbiologists claim that rapid
240 diagnostic is not useful, because studies demonstrating impact of rapid methods on mortality
241 are rare [71-74]. However, in the last years at least two meta-analyses have shown both cost-
242 effectiveness and therapeutic improvements when rapid methods are in place [6,75]. The still
243 disappointing impact on mortality is partly due to the fact that we are still too slow
244 before/after producing results, as virtually all studies had been conducted without optimizing
245 time to bottle load, and importantly mortality was reduced only when rapid methods were
246 accompanied with antimicrobial stewardship [75]. Progress in BSI pathogens diagnostics
247 should thus be based on a bundle approach that includes optimization of the pre-analytical
248 parameters (skin preparation, volume of blood sampled), rapid start of incubation, the use of
249 rapid methods, re-organisation (e.g. 24/7, transportation service) and a close involvement of
250 antimicrobial stewardship teams (Fig. 1). We are now facing organizational issues hindering
251 the advantages of novel technologies by the current working patterns of most laboratories.
252 Although the importance of logistics for the impact of CMBCSs was shown almost two
253 decades ago [62,76] still only 42% of European laboratories insert BC bottles 24 h/day and
254 only 13% start the processing of positive BC bottles around the clock, and less than 5%
255 established 24-h service for validation and transmission of the identification and AST results
256 for BC pathogens to clinicians [13].

257 Substantial improvements in diagnostics can be made without investment in sophisticated and
258 expensive instruments through improving the “microbiologistics”. Workflow modifications,
259 i.e. externalising the BC cabinets and/or by introducing BC diagnostics 24/7 will improve the
260 TTR independent of techniques used in the laboratory [13,62]. It should finally be realized
261 that clinical microbiologists are needed for the patient 24/7 and not only in the daytime from
262 Monday to Friday.

263 Continuous improvement of the whole BSI diagnostic process, based on sampling quality and
264 time to result, should be a priority to improve patient outcome and avoid unnecessary
265 antibiotic treatment. This should be a pivotal guiding line of the European health policies.

266

267 **Transparency declaration**

268 B.L. reports grant from BD and non-financial support from Curetis. M.S. reports ongoing
269 research collaborations with Q-linea and 1928D. E.A.I. is a co-inventor of patent applications
270 in the field of diagnostic microbiology, owned by the University of Münster and licensed to
271 Bruker Daltonik. E.A.I. received a speaker honorarium and reimbursement for congress travel
272 cost from Bruker Daltonik.

273

274 **Funding**

275 No external funding was received for this publication.

276

Table 1. State-of-the-art in microbiological diagnostics of bloodstream infections.

Diagnostic procedure	State-of-the-art^a	Comment	Reference
Blood sampling			
Skin preparation	The contamination rate is <3%, optimally <1%	Contamination is defined as a microorganism recovered from BC bottle that is indeed absent in the patient blood. Most often, contaminations are bacteria from the skin microbiota (e.g., coagulase negative staphylococci, coryneforms). Contamination rate is defined as the number of contaminated samples (whatever the number of positive bottles) per 100 samples (whatever the number of bottles collected). Contamination rate is reduced by compliance with skin preparation best practice, diversion technique, single sampling strategy and prior careful disinfection of bottle's septum	[12]
Bottle-filling (adult patient)	The volume of blood per bottle is 8-10 ml	These two parameters result in a total volume of blood cultured of at least 40 ml to ensure optimal BC sensitivity.	[10,11,16,78]
Number of bottles collected per episode (adult patient)	4-6 bottles are collected (4 acceptable only if all bottles properly filled) Rate of solitary BC (2 bottles) below 10%	For children, refer to article in the current theme review [77].	
Specimen transport	<4h for the majority of BC	With decentralised BC cabinets 2h should be aimed for	

Species identification	The result of species identification is available on the same day of BC positivity	Achievable with MALDI-TOF MS performed directly from positive blood cultures or from short sub-cultures on solid medium. Also achievable with genotypic rapid methods for the most frequent pathogens.	[9,61]
AST	The preliminary or final AST report is available on the same or at latest next day of BC positivity	Achievable by AST initiation on the same day from positive BCs (inoculation of short sub-cultures on solid medium, of microbial pellet, or directly of positive BC broth) instead of application of overnight colonies.	[36]
Reporting of positive results	100% of new positive episodes, both identification and AST, are promptly reported to clinicians	By an active and prompt communication of the index information (e.g., positive flagged BC, Gram stain result or result of the direct ID) through a phone call or an electronic alert. Subsequent information (obtained on Day 1) can be reported using electronic report, except when new information is identified as being critical to patient care (e.g. AST result that shows prompt treatment change is required due to ineffective antibiotic regimen). Index information should preferably be also communicated to an Antibiotic Stewardship Team or an Infectious Diseases department.	[10]

^a State-of-the-art statements represent authors' opinion on what is currently feasible and what should be implemented as requirement for timely and qualitative diagnostics of bloodstream infections

^b AST, antimicrobial susceptibility testing

Table 2. Tools and strategies to control the quality of the pre-analytical phase of blood culture. CMBCS, continuous monitoring blood culture system; CTCB, Centre Toulousain pour le Contrôle en Biologie; ESGBIES, ESCMID Study group for bloodstream infection, endocarditis and sepsis.

Type of progress	Characteristics	Comments	References
1. Use key performance indicators (KPI) to monitor and manage the preanalytical process	<p>KPI defined by a threshold^a to monitor and define continuous quality improvement initiatives. Pre-analytical KPI include:</p> <ul style="list-style-type: none"> - Rate of bottles not reaching the recommended volume of blood (target). This KPI should be linked to items 2, 3, 4, 6 (see below) to reach optimal values and to be optimally monitored - Rate of solitary BC per 24h. This KPI can be improved with item 6 (see below) - Rate of BC with long time to bottle load - Rate of contamination. This KPI is to be linked to 	<ul style="list-style-type: none"> - Should be regularly monitored. - Unsatisfactory results (ie., not reaching minimal acceptable threshold) should trigger actions of improvement (e.g., training, reorganization, etc.) according to e.g. Deming p-d-c-a cycle (plan, do, check, act) - Participating to EQA programmes (item 4, see below) contributes to promote actions of improvements 	[10,14-16]

	items 3, 5 and 6 (see below) to obtain optimal values		
2. CMBCS-incorporated automated systems to monitor the volume of blood	The Bactec FX system (BD, USA) estimates by measuring the CO ₂ produced by red blood cells from at least 25 bottles (batch, ward level)	<ul style="list-style-type: none"> - Mean error of 0.2-0.3 mL - Device accuracy affected by low hematocrit (higher error margin, i.e., 1 mL) - Volume monitoring limited to batch, (no report possible on the reporting of result) 	[14,15,17,79]
	The BC system Virtuo (bioMerieux) estimates using a photometric detection of liquid level for each bottle	<ul style="list-style-type: none"> - Median error [range] of 1.40 [0.82–1.88] and 0.21 [-0.46–0.88] mL compared to the median weight-based volume in aerobic and anaerobic bottles, respectively 	[18]
3. Education	to improve order quality (prescribers)	<ul style="list-style-type: none"> - Order improved when written procedure describing how many BC sets to order is available - Regular feedback to wards that poorly comply order procedure - Communicate on the consequence of not complying 	

		the number of bottles to culture (default of sensitivity)	
	to improve sample quality (samplers)	<ul style="list-style-type: none"> - Training proved efficient to improve the rate of bottle filled with >5 ml of blood - Improvements may be only moderate despite intervention and time-consuming when performed alone but good improvements when combined with regular feedbacks that are facilitated by CMBCS-incorporated automated systems - Needs to improve performance in training methods 	[14,15,80]
4. External quality assessment (EQA) program in Europe	Joint initiatives from CTCB ¹ and ESGBIES ² concerning EQA to assess the volume of blood cultured (rate of bottles not reaching minimum volume of blood KPI and rate of solitary BC per 24 h), in-progress ^b	<ul style="list-style-type: none"> - assesses the current level of quality at a population level (laboratories) - estimates gap to the targeted values according to state-of-art - helps promoting actions of improvement as it shows when a lab performance requires 	[81]

		<p>improvement (low rank and far from target)</p> <ul style="list-style-type: none"> - helps monitoring actions of improvement 	
5. Diversion technique	Diversion tube or device diverts the initial first mL of blood to remove any potentially contaminated skin plug	<ul style="list-style-type: none"> - unambiguously reduces contamination rates - range of rate reduction varies between studies (contamination from 1.5- to 8-fold less) 	[21,22,82]
6. Single sampling strategy	Alternative sample strategy	<ul style="list-style-type: none"> - reduces the rate of solitary BC, thus improves the total volume of blood cultured and sensitivity - reduce the contamination rate 	[11,83,84]

^a Strengthened benchmarks are currently under development. Meanwhile, laboratories can use a reasonable optimal benchmark defined with (for instance) 80% of series or bottles complying with the targets presented in Table 1 (e.g. 80% of bottles filled with 8-10 ml).

^b An EQA focusing on the actual total volume of blood cultured per episode, compliance with bottle filling and compliance with number of bottles ordered, implemented through an ESGBIES/CTCB collaboration and opened to any laboratory, is at the probationary step at time of article revision (first evaluation performed in 2019, 2nd planned in 2020).

Table 3. The impact of different aspects of "microbiologistics" and the introduction of new techniques for rapid species ID and rapid AST on the time from sampling to first report.

In the theoretical scenarios (left) Hospital 1 has not adopted any modern technology while Hospital 2 has adopted many steps to reach a much more rapid process.

In the in practice part (right), monthly median values (in brackets the range of median values during one year) for the BC process in Örebro County, Sweden, has been calculated and illustrates that the impact of introduction 24/7 results in a 9-11 h reduction for time to first results. At the University hospital BCs can be sent through pneumatic tube system to the laboratory for introduction 24/7. At regional hospital A, BCs are brought to the local laboratory and introduced 24/7. Positive cultures are sent to the clinical microbiology laboratory at the University Hospital (45 km) 1-3 times per day at fixed hours. In contrast, at regional hospital B, BCs are brought to the local laboratory and stored in room temperature before being sent to the clinical microbiology laboratory at the University Hospital (45 km) 1-3 times per day, at fixed hours, to start incubation. Importantly, the effect of introduction of BCs 24/7 at regional Hospital A will lead to a much faster median processing time compared to regional hospital B despite the transportation of positive BCs to a central lab.

	Theoretical scenarios		Örebro County, Sweden 2018		
	Hospital 1	Hospital 2	University Hospital Emergency Room	Regional Hospital A	Regional Hospital B

Introduction of BC vials	Daytime 7 days per week	24/7	24/7	24/7	Sent to central lab 1-3 times per day
Processing of BC vials	Daytime 7 days per week	24/7	Daytime 7 days per week	Positive BCs are sent to central lab	Performed at central lab
Species identification	Over night solid agar incubation	Direct MALDI	Direct MALDI	Performed at central lab	Performed at central lab
AST method	AST from subculture	Direct AST with early reading	Direct AST and early reading for Gram negative bacteria daytime	Performed at central lab	Performed at central lab
Sampling to introduction (h)	1-15	1-2	2 (1-3)	3 (2-4)	14 (12-14)
Sampling to positive BC^a (h)	13-27	13-15	na ^b		
Sampling to Gram Stain report (h)	14-28	14-17	na ^b		
Sampling to Species ID (h)	30-44	14-17	38 (29-44)	40 (35-50)	49 (36-63)
Sampling to first AST report (h)	46- 60	20-23	na ^b		

^a The time to positivity was set to 12 h in both examples.

^b na, not analyzed due to lack of confident data stored in the Laboratory Information System

Figure legends.

Figure 1. Summary of all the actions to improve the bloodstream infection pathogen diagnostics. Types of actions belong to 3 complementary axes and actions aim to manage sample quality, times before and after analysis and analytical times. Each action per se is associated with a limited improvement but combination of several actions significantly improves diagnosis. Improvement is maximum when programme include actions on sampling quality, rapid diagnostics and logistics. KPI: Key-performance indicator, EQA: external quality assessment, SSS: single sampling strategy, CMBCS: continuous-monitoring blood culture system, AM Stewardship: antimicrobial stewardship. * rapid tests (e.g., *mecA* detection) may be needed in area of high level of resistance.

Figure 2. Classical approach for the diagnostics of bloodstream infections and current possibilities of process acceleration. In the classical approach, BC bottles are inserted into the continuously monitoring automated BC incubators. In case of growth detection, Gram stain is prepared from the positive BC broth and the result of microscopy is immediately communicated to a clinician. At the same time, positive BC broth is sub-cultivated onto agar plates followed by overnight incubation to grow microbial colonies which are used for identification and antimicrobial susceptibility testing (AST). The novel approaches enable to drastically reduce time to result by reducing transportation time using microbiologistics (inside/outside the laboratory), by using rapid identification and rapid AST methods either from positive broth or after a short subculture.

Figure 3. Advantages and limitations of direct MALDI-TOF MS identification from positive blood cultures, MALDI-TOF MS identification from positive blood cultures using short sub-culture on solid medium, and DNA-based identification from whole blood.

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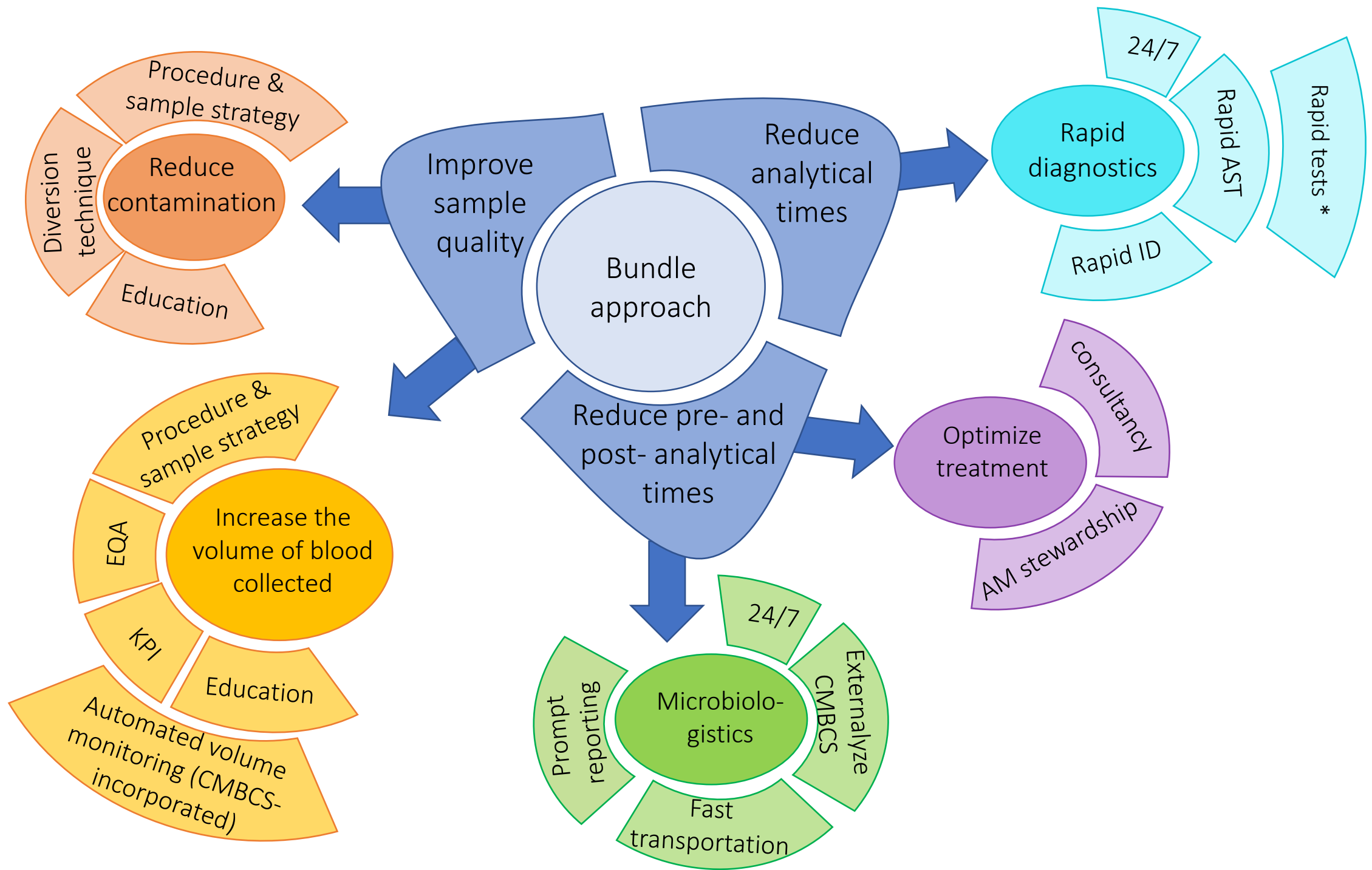
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CLASSICAL APPROACH

1st day

2nd day

3rd day

Transport

Incubation in automated system

Incubation on solid medium

Identification and AST

Sampling

Insertion into automated system

- Detection as positive
- Gram stain
- Sub-cultivation
- Direct AST

Isolated colonies

Final report

ACCELERATED METHODS

Microbiologistics !

MALDI-TOF MS
directly or from short sub-culture

Rapid AST

SUGGESTED APPROACH

Transport

Incubation in automated system

MALDI-TOF MS
directly or
from short subculture

Direct AST
Rapid AST

Result communication and advice on antimicrobial treatment (to ward ± antimicrobial stewardship team)

Direct MALDI-TOF MS identification from positive blood cultures

ADVANTAGES

Very rapid identification
Easy
Low additional cost

LIMITATIONS

Identification difficulties with some species
Confirmation might be needed
Mixed cultures

MALDI-TOF MS identification from positive blood cultures using short sub-culture on solid medium

ADVANTAGES

Rapid identification
Very easy
Easy to integrate in lab workflow
Confirmation usually not needed

LIMITATIONS

Slow-growing organisms
Mixed cultures

Molecular methods for identification from positive blood cultures

ADVANTAGES

Rapid identification
Easy

LIMITATIONS

High cost
Confirmation needed
Limited pathogen panels

DNA-based identification from whole blood

ADVANTAGES

Time advantage compared to culture
Advantage in pathogen detection under antibiotics

LIMITATIONS

Limited number of pathogens in panel assays
Relatively low sensitivity (only 1-5 ml blood sampled)
Resistance detection \neq susceptibility testing
Clinical relevance of DNAemia ?
High workload
Highly experienced staff required
High cost
Analytical cut-offs set by some manufacturers
Does not substitute culture-based testing