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ORIGINAL ARTICLE

Reduced length of hospital stay through a point of care placed automated blood culture instrument

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Abstract Early appropriate antimicrobial treatment of patients with sepsis has a large impact on clinical outcome. To enable prompt and efficient processing of blood cultures, the inoculated vials should be placed into an automated continuously monitoring blood culture system immediately after sampling. We placed an extra BACTEC FX instrument at the emergency department of our hospital and validated the twice-daily re-entering of ongoing vials from this instrument into the BACTEC FX at the laboratory. We subsequently assessed the benefits of shortening the transport time between sampling and monitored incubation of blood culture vials by comparing the turnaround times of positive blood cultures from emergency department patients with a historical control group. Re-entering ongoing vials within 2 h raised no technical problems with the BACTEC FX and did not increase the risk of false-negative culture results. The decreased transport time resulted in significantly earlier available Gram stain results for a large proportion of patients in the intervention group and a significant shortening of the median total turnaround time to less than 48 h. The median length of hospital stay shortened by 1 day. Immediate entering of blood culture vials into a point of care placed BACTEC FX instrument and subsequent efficient processing enables earlier decision-making regarding antimicrobial treatment, preventing the development of antimicrobial resistance and reducing healthcare costs.

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Introduction

For patients suspected of bacteraemia or sepsis, the processing of blood cultures needs to be prompt and efficient to enable escalation or de-escalation of the therapy, which will improve clinical outcome and reduce resistance development risks and costs, in line with current antimicrobial stewardship protocols [1–4]. Immediate entry of blood culture vials into an automated continuous-monitoring blood culture system outside laboratory operating hours is usually not possible. Previous studies showed that storing the vials at room temperature lengthened the time to positivity, and preincubation in a non-monitored incubator gave false-negative results [5, 6].

To shorten the time between sampling and positivity of blood cultures for a category of seriously ill patients, we placed a BD BACTEC FX blood culturing instrument at the emergency department (ED) of our hospital and assessed the impact on Gram stain results availability, mortality and length of hospital stay. We also explored the technical and microbiological limits of re-entering blood culture vials into a BACTEC FX instrument in the laboratory after initial incubation in the BACTEC instrument at the ED.

Materials and methods

Setting

Our microbiology laboratory uses the automated blood culture monitoring system BD BACTEC™ FX (BD Diagnostics, Erembodegem, Belgium). Until the beginning of 2015, blood cultures collected during the day on hospital wards, in intensive care units (ICUs) and the ED were transported during several daily rounds to the laboratory, where they were registered and entered into the BACTEC system. Blood culture

vials collected outside laboratory opening hours (between 8:00 a.m. and 5:00 p.m.) were kept at room temperature until BACTEC incubation.

In March 2015, an extra BACTEC FX instrument was placed at the ED and connected to the same EpiCenter software module as the instruments in the laboratory. ED staff was trained basically to place inoculated vials into the system as soon as possible after sampling, 24/7. Since then, each day at 8:00 a.m. and 2:00 p.m., a laboratory technician picks up all vials from the ED instrument. After registration, vials signalled as positive are processed immediately and ongoing vials are re-entered into the BACTEC system at the laboratory accompanied by a proper identification. This way, sufficient BACTEC capacity is ensured at the ED and ongoing vials re-entered into the laboratory BACTEC which become positive during the day can be processed immediately. Prior to applying this method, we performed a re-entry validation.

Validation of re-entering ongoing vials

The BACTEC signals positive vials by indicator lights, audible alarm and in the EpiCenter software. To ensure optimal performance, the manufacturer recommends not to move any ongoing vials. According to their instructions, a maximum of five measurements may be missed. If a reading gap longer than 40 min caused by power failure occurs, readings start anew after power has been restored. Ongoing vials should, therefore, be removed and subcultured before being replaced in order to avoid false-negative signalling. Ongoing vials removed for any other reason should be replaced within 20 min to avoid too much cooling off. Re-entry is possible up until 5 h, after which all data are discarded [7].

In our setting, unloading vials at the ED, registration and re-entry at the laboratory takes longer than 20 min. Because BD does not technically approve this new methodology, we performed four experiments to validate the re-entry of ongoing blood culture vials after longer transport time windows and to determine the false-negativity rate as a result of our practice. We used blood culture vials BD BACTEC™ Plus Aerobic/F and BD BACTEC™ Lytic/10 Anaerobic/F.

(i) To validate re-entry after 20 min, eight uninoculated blood culture sets were placed in a BACTEC instrument. After a few minutes, the vials were removed and kept for, respectively, 20, 30, 60, 120 and 300 min, after which each set was re-entered into a second instrument. System messages were recorded.

(ii) To assess the impact of varying transport times on the time to detection (TTD; time between BACTEC entry and positive signal), culture sets were inoculated with human donor blood and strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 10211 and *Bacteroides fragilis* ATCC 23745, respectively [final concentrations of 3–50 colony-forming units (CFU)/ml]. All vials were placed in a BACTEC instrument, removed after 4 h, kept for, respectively, 20, 30, 60 and 120 min and re-entered into a second instrument. For each strain, a set of vials which was not 'transported' was used as a control. The TTDs of transported vials were compared to the controls.

(iii) To assess the impact on the TTD of less than five measurements at the beginning of BACTEC incubation, culture sets were inoculated with human donor blood and strains of *Escherichia coli* ATCC 25922 and *Streptococcus pneumoniae* ATCC 49619 (final concentrations of 6 and 19 CFU/ml). All vials were placed in a BACTEC instrument. Sets were removed after, respectively, 20, 40, 60 and 80 min, kept for 60 min and re-entered into a second instrument. For each strain, a set of control vials was processed. The TTDs of transported vials were compared to the controls.

(iv) To determine the risk of false-negative culture results, we blindly subcultured a large series of blood cultures which, after initial incubation in the ED BACTEC, had been transferred to the laboratory BACTEC and called negative after 5 days of incubation. One drop of broth from each aerobic vial was plated onto chocolate agar (bioMérieux, Marcy l'Etoile, France), from each anaerobic vial onto Schaedler's agar (Mediaproducts, Groningen, The Netherlands). Plates were incubated at 35 °C in 5 % CO_2-enriched atmosphere and in anaerobic conditions, respectively, for 5–7 days.

Data collection and definitions

From July up to and including December 2015, we prospectively collected data of all blood cultures sent in from patients visiting the ED (intervention group ED2015). Of the culture vials signalled positive by the BACTEC FX system within 5 days of incubation, patient and culture data were compared to a historical control group of positive ED cultures performed in the same period in 2014, without a BACTEC FX instrument in place at the ED yet (conventional group ED2014). In both groups, only samples collected in BD BACTEC™ Plus Aerobic/F and BD BACTEC™ Lytic/10 Anaerobic/F culture vials were included. Cultures without a known sampling time were excluded. Patients could be included more than once, since each new ED visit and subsequent positive blood cultures were considered to represent a new septicaemic episode.

Of each culture, we recorded patient data, culture results, sampling date and time, the TTD and the time intervals between sampling and, respectively, entry into the BACTEC FX, a positive signal, Gram stain results, identification (ID) results and antimicrobial susceptibility testing (AST) results.

The TTD of the first positive vial was used in the analysis. The moment Gram stain results would be available was defined as 30 min after a positive signal during laboratory opening hours (so at the latest at 5:30 p.m.) or on the following day at 9 a.m. if the vial became positive during lab closure. If Gram stain results were known before noon, a subculture was made of the blood culture medium, of which bacterial ID results, obtained through matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), were supposed to be ready at 4 p.m. the same day. If Gram stain results became known later than 12 a.m., a subculture was made and MALDI-TOF MS ID results would be available the next day at 10 a.m. Antimicrobial susceptibility tests started from subcultures were defined to be available at 8 a.m. the next day.

Isolates were considered to be clinically relevant or classified as contaminants according to international guidelines and additional assessment by a clinical microbiologist [8].

Patient data comprised gender, age, length of hospital stay and mortality, calculated as death within 30 days of hospital admission or in-hospital death if the admission period extended beyond 30 days [9]. Cultures containing isolates considered as contaminants were excluded from mortality and length of hospital stay analyses.

Analysis and statistics

SPSS software version 22.0 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) was used for all statistical analyses, with the Mann–Whitney *U*-test (MW) for categorical data and Pearson χ^2 test for dichotomous data. $p \leq 0.05$ was considered statistically significant.

Sample size for positive blood cultures in each study group was calculated by comparing the data of July 2014 and July 2015. A sample size of 342 blood cultures in each group would detect a difference between the median intervals sampling to Gram stain results of at least 3 h with a power of 80 % ($\alpha = 0.05$).

Results

Validation of re-entering ongoing vials

(i) The BACTEC system accepted re-entry of blood culture sets after virtual transport intervals of 20, 30, 60 and 120 min without any error messages. Re-entry after 5 h triggered the message: "*Scanned vial has been out of the instrument for longer than recommended reentry and if returned it's protocol will be restarted.*"

(ii) After 4 h of BACTEC incubation and virtual transport times of 20, 30, 60 and 120 min, TTDs after re-entry ranged from 10.9 to 12.6 h for *Escherichia coli*, 12.8–14.6 h for *Staphylococcus aureus*, 12.7–18.3 h for *Haemophilus influenzae*, 12.5–17.8 h for *Streptococcus pneumoniae* and 23.5–65.0 h for *Bacteroides fragilis*. There were no differences in ranges between aerobic and anaerobic TTDs, or between re-entered vials and controls.

Table 1 Patient demographics

Characteristic	ED2014 ($n = 224$)	ED2015 ($n = 241$)	*p*-Value
Gender			0.136
Male (no., %)	127 (57)	120 (50)	
Female (no., %)	97 (43)	121 (50)	
Age (median years, IQR)	74 (65–82)	72 (63–79)	0.131

IQR interquartile range

Table 2 Isolate distribution in blood cultures between study groups

Isolate group and name[a]	ED2014, no. (%)	ED2015, no. (%)
Enterobacteriaceae	195 (42.8)	222 (43.8)
Escherichia coli	148	167
Klebsiella spp.	30	37
Enterobacter spp.	7	5
Proteus mirabilis	2	6
Citrobacter spp.	6	1
Salmonella spp.	0	5
Serratia spp.	1	1
Morganella morganii	1	0
Non-fermenters	10 (2.2)	9 (1.8)
Pseudomonas aeruginosa	9	7
Acinetobacter spp.	1	1
Brevundimonas diminuta	0	1
Staphylococcus aureus	30 (6.6)	38 (7.5)
Coagulase-negative staphylococci	58 (12.7)	62 (12.2)
Enterococcus spp.	17 (3.7)	21 (4.1)
Streptococcus spp.	86 (18.9)	91 (17.9)
Streptococcus pneumoniae	38	33
Streptococcus agalactiae	5	10
Streptococcus pyogenes	6	4
Other	37	44
Anaerobes	13 (2.9)	21 (4.1)
Bacteroides spp.	4	10
Clostridium spp.	0	7
Other	9	4
Other	21 (4.6)	29 (5.7)
Polymicrobial	26 (5.7)	14 (2.8)

[a] Names determined by MALDI-TOF MS and, if necessary, the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and additional biochemical methods

(iii) After initial incubation of 20, 40, 60 and 80 min, virtual transport time of 60 min and re-entry, TTDs for *Escherichia coli* and *Streptococcus pneumoniae* ranged from 10.6 to 13.0 h and from 12.6 to 46.0 h. There were no differences in ranges between aerobic and anaerobic TTDs, or between re-entered vials and controls.

(iv) In total, 2135 blood culture vials (as a part of 1099 blood cultures) which had been signalled as negative were subcultured. All were negative, except one aerobic vial subculture containing a Gram-negative bacterium identified by 16S rRNA sequencing as *Moraxella osloensis*. Subcultures from the corresponding anaerobic vial and two more culture sets from the same patient remained negative.

Patient data and culture results

In total, 963 positive blood cultures were included, collected from 465 patients with equal gender and age distributions between the study groups (Table 1). The culture results (Table 2) did not differ between the groups (p = 0.482). The distribution of clinically relevant isolates and contaminants was equal as well (Table 3). The median transport time for the conventional group of 11.1 h was shortened to 0.1 h for the intervention group as a result of the direct entry (Table 4). Although the TTD (start of BACTEC to positivity) was longer for ED2015, culture vials were signalled as positive 8 h earlier than before direct entry was possible. Gram stain results were available approximately 6 h earlier (Fig. 1), and, thereby, the percentage of Gram stain information available the day after sampling increased significantly from 52 to 76 %. Mortality in both groups was similar, but the median length of hospital stay was 1 day shorter for the intervention group (Table 4).

Table 3 Distribution of relevant isolates and contaminants in blood cultures between study groups

Isolates	ED2014, no. (%)	ED2015, no. (%)	p-Value
Clinically relevant	406 (89.0)	460 (90.7)	0.383
Contaminants	50 (11.0)	47 (9.3)	

Discussion

The possibility to load blood culture vials into a BACTEC instrument at any moment during the day or night at the ED shortened the transport time to mere minutes. This resulted in Gram stain results being available a day earlier for a large proportion of patients, which is critical for decision-making in antimicrobial treatment. For almost 20 % more of the positive cultures, Gram stain results could be reported to the clinician before 12:00 a.m., increasing the chance of effective follow-up. The median total turnaround time shortened to less than 48 h and the length of hospital stay shortened by 1 day, allowing, in our hospital, a return on investment within 6 months after placement of the extra BACTEC instrument.

With a BACTEC instrument in place at the ED, the most efficient way of obtaining blood culture results is to transport all loaded vials to the laboratory one or twice daily, process

Table 4 Turnaround times, availability of Gram stain results and clinical outcome

Interval (median hours, IQR[a])	ED2014 (n = 456)	ED2015 (n = 507)	p-Value
Sampling - start BACTEC	11.1 (2.4–16.2)	0.1 (0.0–0.4)	<0.001
TTD[b]	10.7 (7.0–16.9)	13.0 (10.7–19.9)	<0.001
Sampling - BACTEC positive signal	21.5 (15.5–26.7)	13.5 (11.2–20.8)	<0.001
Sampling - Gram stain results	25.4 (21.0–37.6)	19.8 (15.1–24.3)	<0.001
Sampling - ID results	40.1 (29.0–45.0)	30.0 (24.5–40.8)	<0.001
Sampling - AST results	58.2 (45.1–63.0)	47.0 (40.5–59.8)	<0.001
Percentage of Gram stain results (%, no.)			
Available next day[c]	52.0 (237)	76.1 (386)	<0.001
Available next day before noon[c]	32.5 (148)	51.5 (261)	<0.001
Outcome	ED2014 (n = 186)	ED2015 (n = 210)	p-Value
Mortality (no., %)	18 (9.7)	25 (11.9)	0.477
LOS[d] (median days, IQR [a])	7 (5–13)	6 (4–11)	0.024

[a] Interquartile range

[b] Time between entering BACTEC and positivity

[c] The day following sampling

[d] Length of hospital stay

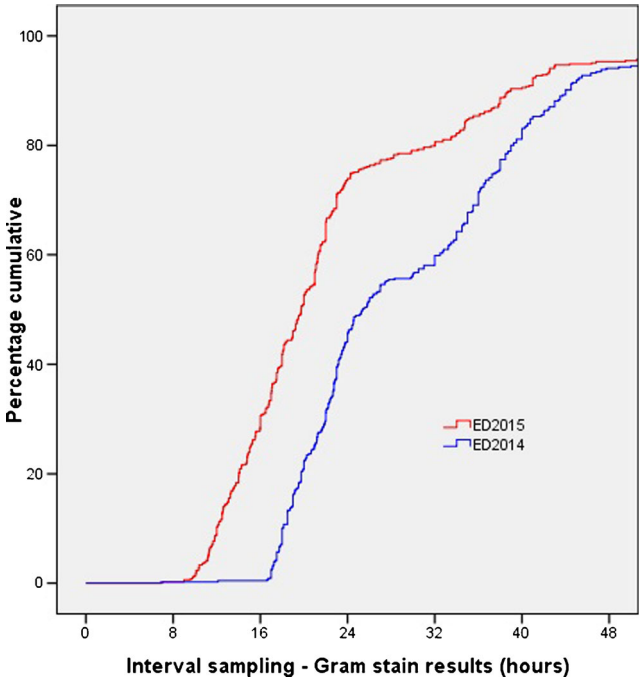

Fig. 1 Time between sampling and availability of Gram stain results for the study groups ED2015 (intervention group) and ED2014 (control group)

positive vials immediately and re-enter ongoing vials into a laboratory BACTEC until positivity or final negative signal. This way, the positive-to-removal time will be as short as possible [10].

None of our validation experiments rendered false-negative cultures, except for one subcultured vial containing *Moraxella osloensis*, which was retrospectively considered to have been a contaminant. With one positive result in 2135 blindly subcultured vials, our expected false-negative rate through replacing vials lies well below the performance of 0.2–0.3 % false-negatives claimed by the manufacturer for uninterrupted incubation [11].

Others experimented with preincubation of blood culture vials in non-monitoring incubators at 35–37 °C outside the laboratory [6, 12, 13]. Although TTDs decreased significantly, false-negative rates due to fully grown bacteria increased, requiring visual inspection and subculturing of the vials on arrival in the laboratory.

Gram stain results still remain critical in the management of sepsis patients and, therefore, the first focus in shortening the turnaround time should be on the transport time of blood cultures. By direct entry into a continuous-monitoring system, Gram stain results will be available significantly earlier, with a negligible risk of false-negative results. As a result, the length of hospital stay will shorten and resistance will be prevented, consistent with current views regarding antimicrobial stewardship.

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Compliance with ethical standards

Funding There was no funding.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and informed consent All procedures performed in our study involving human participants were in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

For this type of study, formal consent is not required.

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