Major pathogen microorganisms except yeasts can be detected from blood cultures within the first three days of incubation: A two-year study from a University Hospital

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Abstract. The knowledge of the expected time-to-positivity (TTP) of blood cultures by major pathogens is essential both clinically and economically. To this end, we conducted the present two-year study in our Institution, aiming to assess the TTP of all the major microorganisms including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetoacter baumannii*, Enterococcii spp, *Staphylococcus aureus* and yeasts, to determine whether a 3-day interval is sufficient for their detection. The TTP for each case of strain isolation per patient was determined as the TTP of the first bottle among a set of bottles collected within the same period of time to be flagged as positive per patient. Based on our results, almost all major Gram-negative (99.30%), Gram-positive microbia (99.01%) and yeasts (98.85%) were detected within the first 5-days of incubation, leading to the solid conclusion that a 5-day period of incubation is adequate to detect almost all the major routine pathogens. By contrast, when a 3-day period was examined acceptable results were only found for Gram-negative (98.33%) and Gram-positive (98.51%) microbia. A significant proportion of yeasts (8.05%) could not be detected within this time frame. Therefore, regarding the yeasts, a 3-day incubation period cannot be considered as adequate and is not advocated.

Introduction

It is generally accepted that with the use of modern continuous- monitoring automated blood culture (BC) instruments, less than 5 days of incubation are required for a positive result (1,2). Moreover, there are reports that even a 3-day period of incubation is sufficient (3,4). A positive BC result, along with the time-to-positivity (TTP) can be influenced by a series of independent factors, such as the different patients populations, the number of BC sets collected, the timing of BC collection, the volume of blood drawn, the overall collection procedure applied (the kind and the way skin disinfectants are used, and the experience of phlebotomists), along with the time elapsed from collection to the onset of incubation and the storage conditions during this period. These parameters differ, not only among different geographical areas, but also among hospitals within the same area.

The knowledge of the expected TTP of BCs by major pathogens is essential both clinically and economically. To this end, we conducted the present study in our Institution, aiming to assess the TTP of the major microorganisms and to determine whether a 3-day interval is sufficient for their detection. To the best of our knowledge, this is the first such investigation conducted in the area of Crete, Greece.

Materials and methods

Our Institution is a referral 750-bed tertiary University Hospital in our region and performs an average of 14,749 BCs per year. A 7-day incubation protocol is applied. In the present study, positive BC results regarding clinically significant microorganisms over a period of 2 years (1/11/2014 - 31/10/2016) were retrospectively analyzed. In particular, the major Gram-negative bacteria including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetoacter baumannii* along with major Gram-positive microbia including Enterococcii spp and *Staphylococcus aureus* (*S. aureus),* were processed. Additionally, any yeasts that were fully identified over this period were also included. The TTP for each case of strain isolation per patient was determined as the TTP of the first bottle among a set of bottles collected within the same period of time (same day) to be flagged as positive per patient. Days were calculated as full 24-h frames. For example, if a positive result was obtained at or after 24.1 h of incubation it was considered to

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Table I. Time-to-positivity (in h) and percentages (%) of positive blood cultures per day of incubation.

Microorganism	Mean	Min	Max	D1 % (N/Ntot)	D2 % (N/Ntot)	D3 % (N/Ntot)	≥D4 h % (N/Ntot)	NoB	NoP
E. coli	17.8	1.9	179.5	89.71 (157/175)	2.86 (5/175)	2.29 (4/175)	5.14 (9/175)	175	157
A. baumannii	12.1	4.3	51.6	97.19 (138/142)	2.11 (3/142)	0.70 (1/142)	0.00 (0/142)	142	84
P. aeruginosa	17.6	4.6	85.0	91.13 (113/124)	6.45 (8/124)	1.61 (2/124)	0.81 (1/124)	124	82
K. pneumoniae	13.1	2.2	86.9	95.20 (119/125)	2.40 (3/125)	1.60 (2/125)	0.80 (1/125)	125	87
K. oxytoca	12.0	4.6	32.2	100.00 (18/18)	0.00 (0/18)	0.00 (0/18)	0.00 (0/18)	18	18
P. mirabilis	13.0	6.5	25.7	95.45 (21/22)	4.55 (1/22)	0.00 (0/22)	0.00 (0/22)	22	20
S. marcescens	20.0	4.1	139	81.40 (35/43)	11.61 (5/43)	4.66 (2/43)	2.33 (1/43)	43	24
E. cloacae	12.9	4.8	39.8	91.11 (41/45)	8.89 (4/45)	0.00 (0/45)	0.00 (0/45)	45	36
S. maltophilia	19.3	9.4	63.4	88.00 (22/25)	8.00 (2/25)	4.00 (1/25)	0.00 (0/25)	25	13
S. aureus	20.9	4.1	146.2	81.12 (73/90)	12.22 (11/90)	3.33 (3/90)	3.33 (3/90)	90	65
E. faecalis	13.4	2.2	29.5	95.00 (57/60)	5.00 (3/60)	0.00 (0/60)	0.00 (0/60)	60	50
E. faecium	14.8	5.5	55.2	91.84 (45/49)	6.12 (3/49)	2.04 (1/49)	0.00 (0/49)	49	43
E. gallinarum	17.4	15.1	19.7	100.00 (2/2)	0.00 (0/2)	0.00 (0/2)	0.00 (0/2)	2	2
E. durans	13.0	13.0	13.0	100.00 (1/1)	0.00 (0/1)	0.00 (0/1)	0.00 (0/1)	1	1
C. parapsilosis	35.6	9.1	116.2	22.22 (10/45)	62.23 (28/45)	13.33 (6/45)	2.22 (1/45)	45	22
C. albicans	31.1	2.2	61.4	39.77 (8/26)	53.85 (14/26)	15.38 (4/26)	0.00 (0/26)	26	20
C. tropicalis	17.5	9.4	28.6	75.00 (6/8)	25.00 (2/8)	0.00 (0/8)	0.00 (0/8)	8	7
C. glabrata	79.9	24.5	121.4	0.00 (0/8)	12.50 (1/8)	12.50 (1/8)	75.00 (6/8)	8	4

D1: first day of incubation, D2: second day of incubation, D3: third day of incubation, and D4: fourth day of incubation. N, number; Ntot, total number; NoB, number of bottles, NoP, number of patients; *E. coli*, *Escherichia coli*; *A. baumannii*, *Acinetobacter baumannii*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. pneumoniae*, *Klebsiella pneumoniae*; *K. oxytoca*, *Klebsiella oxytoca*; *P. mirabilis*, *Proteus mirabilis*; *S. marcescens*, *Serratia marcescens*; *E. cloacae*, *Enterobacter cloacae*; *S. maltophilia*, *Stenotrophomonas maltophilia*; *S. aureus*, *Staphylococcus aureus*; *E. faecalis*, *Enterococcus faecalis*; *E. faecium*, *Enterococcus faecium*; *E. gallinarum*, *Enterococcus gallinarum*; *E. durans*, *Enterococcus durans*; *C. parapsilosis*, *Candida parapsilosis*; *C. albicans*, *Candida albicans*; *C. tropicalis*, *Candida tropicalis*; *C. glabrata*, *Candida glabrata*.

be positive on day 2. Only bottles with blood inocula were included. The BCs were performed using the BacT/ALERT instrument and identification was performed using Vitek-2 (both from BioMérieux, Marcy-l'Étoile, France).

Results

Pathogens. Results regarding each particular kind of pathogen are shown in Table I.

Gram-negative isolates. Regarding the Gram-negative isolates, 719 positive bottles taken from 521 different patients were analyzed. The distribution of these 719 positive bottles regarding each Gram-negative species is shown in Table I. Table I also provides the time-to-positivity (in h) and percentages (%) of positive blood cultures per day of incubation regarding each Gram-negative species. As shown in Table I, over 9 out of 10 of these bottles (92.35%; 664/719) were flagged positive within the first 24 h. For the second and third day the ratios were 4.31% (31/719) and 1.69% (12/719), respectively. Four or more days of incubation were necessary for 1.69% of the cases (12/719). Of the 12 isolates, 6 or more days of incubation were necessary for 5 isolates, all *Escherichia coli*. Therefore, of the 719 Gram-negative isolates 98.33% (707/719) were flagged positive within the first 3 days and 99.30% (714/719) within the first 5 days of incubation.

Gram-positive isolates. Regarding the Gram-positive isolates, 202 positive bottles taken from 161 different patients were analyzed. The distribution of the 202 positive bottles regarding each Gram-positive species is shown in Table I. Table I also provides the time-to-positivity (in h) and percentages (%) of positive blood cultures per day of incubation regarding each Gram-positive species. As shown in Table I, a percentage of 88.12% of the bottles (178/202) was flagged positive within the first 24 h followed by 8.41% (17/2012) and 1.98 (4/202) for the second and third day, respectively. Four or more days of incubation were necessary for only three isolates (1.48%; 3/202). All three isolates were *S. aureus*. One was isolated on day 4 and two were isolated on day 6. Therefore, of the 202 Gram-positive isolates, 98.51% (199/202) were flagged positive within the first 3 days and 99.01% (200/202) within the first 5 days of incubation.

Yeasts. Regarding the yeasts, 87 positive bottles taken from 53 patients were analyzed. The distribution of the 87 positive bottles regarding each yeast species is shown in Table I. Table I also provides the time-to-positivity (in h) and percentages (%) of positive blood cultures per day of incubation regarding each yeast species. As shown in Table I, only 27.59% of the bottles (24/87) were flagged positive within the first 24 h, whereas the majority of the bottles were designated positive on the second day (51.72%; 45/87) followed by 12.64% (11/87) on the third day. A significant ratio (8.05%; 7/87) needed 4 or more days of incubation. Of the 7 yeasts one *Candida parapsilosis* and four *Candida glabrata* (*C. glabrata*) were isolated on day 4 and only one *C. glabrata* was isolated on day 6. Therefore, 91.95% (80/87) of the *Candida* sp. isolates were detected within the first 3 days and 98.85% (86/87) were isolated within the first 5 days of incubation.

Discussion

In the present study, we assessed the TTP of the BC bottles over a period of two years in our hospital, in order to determine i) the expected TTP of each major pathogen, and ii) whether a 3- or a 5-day incubation period is adequate to detect all major routine pathogens when blood samples were incubated. To the best of our knowledge, this is the first such study conducted in the area of Crete, Greece.

Apart from the retrospective nature of the present study, there are two additional limitations: i) It included only those bottles that had blood inocula and not bottles with other normally sterile body fluid inocula, and ii) it was restricted only to major pathogens and did not include the whole spectrum of microbia. These drawbacks restrain us from generalizing conclusions obtained in this study.

Based on our results, almost all the major Gram-negative (99.30%) and Gram-positive (99.01%) microbia were detected within the first 5-days of incubation. Moreover, almost all the yeasts (98.85%) were detected within this time frame, leading to the solid conclusion that a 5-day period of incubation is adequate to detect almost any major routine pathogens. These results are consistent with those of previous reports, thus verifying our conclusions (4-6).

On the other hand, when a 3-day period was examined, acceptable results were only found for Gram-negative (98.33%) and Gram-positive (98.51%) microbia. A significant proportion of yeasts (8.05%) were not detected within this time frame. Since the presence of yeasts in the blood of a patient is a very urgent situation and represents a high risk of mortality such a ratio cannot be ignored. Therefore, a 3-day incubation period cannot be considered as adequate and is not advocated. It can only be carefully applied in cases of emergency and limited instrument capacity, providing that the time of incubation for the bottles from patients with a prospective fungemia can be adjusted to a routine 5- or even 7-day period.

Nevertheless, additional studies such as the present one from different geographical areas are strongly advocated in order to strengthen the applicability of conclusions obtained herein.

References

1. Cornish N, Kirkley BA, Easley KA and Washington JA: Reassessment of the incubation time in a controlled clinical comparison of the BacT/Alert aerobic FAN bottle and standard anaerobic bottle used aerobically for the detection of bloodstream infections. Diagn Microbiol Infect Dis 32: 1-7, 1998.
2. Reisner BS and Woods GL: Times to detection of bacteria and yeasts in BACTEC 9240 blood culture bottles. J Clin Microbiol 37: 2024-2026, 1999.
3. Bourbeau PP and Pohlman JK: Three days of incubation may be sufficient for routine blood cultures with BacT/Alert FAN blood culture bottles. J Clin Microbiol 39: 2079-2082, 2001.
4. Bourbeau PP and Foltzer M: Routine incubation of BacT/ALERT FA and FN blood culture bottles for more than 3 days may not be necessary. J Clin Microbiol 43: 2506-2509, 2005.
5. Wilson ML, Mirrett S, Reller LB, Weinstein MP and Reimer LG: Recovery of clinically important microorganisms from the BacT/Alert blood culture system does not require testing for seven days. Diagn Microbiol Infect Dis 16: 31-34, 1993.
6. Doern GV, Brueggemann AB, Dunne WM, Jenkins SG, Halstead DC and McLaughlin JC: Four-day incubation period for blood culture bottles processed with the Difco ESP blood culture system. J Clin Microbiol 35: 1290-1292, 1997.