

Original Research Article

PERFORMANCE OF FIVE-DAY AUTOMATED AEROBIC BLOOD CULTURE INCUBATION AND THE UTILITY OF TERMINAL SUBCULTURE

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ABSTRACT

Background: Automated blood-culture systems permit continuous monitoring and early detection of bloodstream pathogens; however, the optimal incubation duration remains debated. This study evaluated whether five days of aerobic incubation ensured adequate organism recovery and determined the additional diagnostic yield of terminal subculture from bottles remaining instrument-negative after 120 hours.

Materials and Methods: This prospective laboratory-based observational study included 1,878 consecutive aerobic blood-culture bottles processed using an automated blood-culture system at a tertiary-care hospital. Bottles were incubated continuously for five days. Positive bottles underwent Gram staining, subculture, identification and antimicrobial susceptibility testing. Bottles remaining negative after 120 hours were subjected to terminal subculture on blood, chocolate and MacConkey agar. Primary outcomes were clinically significant terminal-subculture recovery and diagnostic performance of the five-day protocol. Time to positivity, organism distribution, contamination and associated pre-analytical factors were also assessed.

Results: Of 1,878 bottles, 287 (15.3%) flagged positive, comprising 245 clinically significant isolates and 42 contaminants. Among clinically significant isolates, 95.1% were detected within 48 hours, 98.8% within 72 hours and all by 96 hours. Enterobacterales were predominant, accounting for 47.3% of pathogens. Pathogens had a shorter median time to positivity than contaminants (17.6 versus 58.6 hours; $p < 0.001$). Terminal subculture of 1,591 automated-negative bottles yielded seven organisms (0.44%), of which only two (0.13%) were clinically significant. The five-day protocol demonstrated 99.19% sensitivity, 99.87% negative predictive value and 97.66% accuracy. Prior antimicrobial therapy was associated with reduced automated positivity (11.6% versus 18.6%; $p < 0.001$), while delayed loading was associated with greater terminal-subculture recovery ($p = 0.012$).

Conclusion: Five-day automated aerobic incubation provided highly reliable recovery of clinically significant organisms. Routine terminal subculture produced minimal additional diagnostic yield. Retention of the 120-hour protocol appears prudent, although prompt loading, pre-antimicrobial collection and appropriate sampling remain essential.

Keywords: automated blood culture; aerobic incubation; bloodstream infection; terminal subculture; time to positivity.

INTRODUCTION

Bloodstream infections (BSIs) are important causes of sepsis, prolonged hospitalization and death.

Population-based estimates suggest that BSIs account for more than 1.7 million episodes and approximately 236,000 deaths annually across North America and Europe alone.^[1] Rapid and reliable

detection of the causative organism is essential for initiating targeted antimicrobial therapy, facilitating antimicrobial de-escalation and improving patient outcomes.

Blood culture remains the reference standard for diagnosing bacteraemia and fungemia. Its diagnostic yield is influenced by the volume and number of blood samples collected, timing of collection, prior antimicrobial exposure, transport delay, bottle formulation and incubation conditions.^[2] Automated continuous-monitoring systems have improved blood-culture diagnostics by detecting microbial growth through changes in carbon dioxide production, fluorescence or colorimetric signals. These systems provide continuous incubation and earlier positivity alerts while reducing manual handling.

A five-day (120-hour) incubation period is conventionally recommended for routine automated blood cultures.^[2] This duration is intended to recover most clinically significant bacteria and yeasts, including relatively slow-growing or fastidious organisms. Nevertheless, improvements in culture media, antimicrobial-neutralizing resins and detection algorithms have raised questions regarding the minimum incubation period necessary for optimal recovery. Earlier evaluations of BacT/ALERT bottles found that approximately 97.5% of clinically significant isolates were detected within the first three days.^[3,4] Furthermore, prolonged incubation or routine terminal subculture has generally produced few additional clinically important isolates and may preferentially recover contaminants such as *Cutibacterium* species or coagulase-negative staphylococci.^[5,6]

Recent findings remain system-dependent. With BacT/ALERT VIRTUO, only 0.1% of bottles flagged positive after 96 hours, supporting a four-day protocol.^[7] Conversely, an evaluation of the BD BACTEC FX found that 2% of positive cultures flagged after 96 hours and that some late isolates were clinically significant, supporting retention of the full five-day period.^[8] Therefore, incubation protocols should not be transferred uncritically between platforms. Local validation of the five-day aerobic incubation protocol is necessary to determine whether it ensures optimal organism recovery while avoiding unnecessary extended incubation, delayed final reporting and inefficient use of instrument capacity.

MATERIALS AND METHODS

Study design and setting: This prospective, laboratory-based observational study was conducted in the Department of Microbiology of a tertiary-care teaching hospital over a period of 12 months, from June 2024 to May 2025. The study evaluated whether five days (120 hours) of aerobic incubation in the BacT/ALERT VIRTUO automated blood-culture system provided optimal recovery of clinically significant bacteria and yeasts. All eligible aerobic

blood-culture bottles received during the study period were enrolled consecutively.

Eligibility criteria: Aerobic blood-culture bottles collected from neonates (0–28 days), older paediatric patients (>28 days to <18 years) and adults (≥18 years) with clinically suspected bloodstream infection were included in the study. Bottles with improper identification, visible leakage or damage, inadequate or excessive blood volume, prolonged transportation without validated pre-incubation, or missing collection information were excluded. Bottles specifically submitted for mycobacterial or mould culture and those requiring extended incubation because of suspected unusual pathogens were also excluded. When multiple bottles were collected from the same patient during a single suspected septic episode, each bottle was evaluated for laboratory performance, while patient-level interpretation was based on the complete culture set.

Sample Size: The sample size was calculated using the single-population proportion formula. Based on previous evidence, it was anticipated that approximately 1% of aerobic blood-culture bottles reported negative after five days of automated incubation might demonstrate growth on terminal subculture. At a 95% confidence level and an absolute precision of 0.5%, the minimum required number of culture-negative bottles was 1,521. After allowing for approximately 10% exclusions due to inadequate blood volume, bottle leakage, labelling errors or protocol deviations, the adjusted sample size was 1,690 culture-negative bottles. Assuming that approximately 90% of all blood-culture bottles would remain negative after five days, at least 1,878 bottles were required to be enrolled. Accordingly, the final target sample size was rounded to 2,000 consecutive aerobic blood-culture bottles. The individual aerobic bottle was considered the primary unit of laboratory analysis, while repeated bottles from the same patient were accounted for separately during patient-level clinical interpretation.

Specimen collection and transportation: Blood cultures were requested for patients with clinically suspected bloodstream infection, including sepsis or septic shock, unexplained fever or hypothermia with chills, hypotension or acute organ dysfunction, febrile neutropenia, suspected catheter-related bloodstream infection and infective endocarditis. Blood was collected aseptically, preferably before antimicrobial therapy, without delaying urgent treatment in critically ill patients. For suspected sepsis in adults, two blood-culture sets were collected from separate peripheral venepuncture sites during the same clinical episode. Each set ordinarily comprised one aerobic and one anaerobic bottle; however, only aerobic bottles were included in the present analysis. In paediatric patients, one or two age- and weight-appropriate sets were collected according to the clinical condition and available blood volume. For suspected infective endocarditis, three sets were obtained from separate peripheral venepuncture sites, preferably at approximately 30-

minute intervals before antimicrobial administration; collection was not timed to coincide with fever peaks. Follow-up cultures were repeated after 48–72 hours, where clinically indicated, to document clearance of bacteraemia.

The venepuncture site was disinfected using 2% chlorhexidine in 70% isopropyl alcohol and allowed to dry completely. The rubber septum of each bottle was also disinfected before inoculation. Approximately 8–10 mL of blood was inoculated into each adult bottle. For paediatric bottles, the inoculated volume was determined by age and body weight, generally 0.5–1 mL in neonates and young infants and 1–4 mL in older children, without exceeding the recommended proportion of the estimated circulating blood volume. Bottles were labelled with the patient's identification, collection date and time, anatomical site and set number. They were transported upright to the microbiology laboratory at room temperature and loaded into the automated blood-culture system as early as possible, preferably within two hours. Bottles were neither refrigerated nor pre-incubated outside the automated system.

Automated incubation and processing: All bottles were loaded into the BacT/ALERT VIRTUO system and incubated continuously at approximately 35–37°C for five days. The instrument monitored microbial growth by detecting colorimetric changes associated with carbon dioxide production. Bottles flagged positive at any time during the 120-hour period were removed immediately. A Gram-stained smear was prepared directly from the positive broth, and the preliminary result was communicated promptly to the treating unit. The broth was subcultured onto 5% sheep blood agar, chocolate agar and MacConkey agar. Additional media were used when indicated by the Gram-stain morphology. The exact time to positivity, expressed in hours from loading to the positive signal, was retrieved from the instrument database.

Terminal subculture of negative bottles: Aerobic bottles that remained instrument-negative after 120 hours were subjected to terminal blind subculture to determine whether viable organisms had escaped automated detection. After disinfecting the bottle septum, broth was withdrawn aseptically using a sterile needle and syringe and inoculated onto blood agar, chocolate agar and MacConkey agar. Blood and MacConkey agar plates were incubated aerobically at 35–37°C, while chocolate agar was incubated in 5% carbon dioxide. Plates were examined after 24 and 48 hours. Any growth obtained on terminal subculture was correlated with Gram staining of the bottle broth and confirmed by repeat subculture where necessary to exclude laboratory contamination.

Identification and antimicrobial susceptibility testing: Organisms were identified using standard colony morphology, Gram staining, biochemical reactions and the automated identification system

available in the laboratory, such as VITEK 2 or MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was performed by automated or Kirby–Bauer disc-diffusion methods and interpreted according to the contemporaneous Clinical and Laboratory Standards Institute guidelines. Routine internal quality-control strains were included for identification and susceptibility-testing procedures.

Outcome measures and interpretation: The primary outcome was the proportion of bottles reported negative by the automated system after five days that yielded a clinically significant organism on terminal subculture. Secondary outcomes included overall culture positivity, distribution of recovered organisms, time-to-positivity pattern, proportion detected on each incubation day, contamination frequency and false-negative rate of the five-day protocol. Isolates such as *Staphylococcus aureus*, Enterobacterales, *Pseudomonas aeruginosa*, β -haemolytic streptococci and yeasts were generally considered clinically significant. Common skin flora were classified as pathogens, contaminants or indeterminate isolates after considering the number of positive bottles, recovery from repeated cultures, collection site and corresponding clinical findings.

Statistical analysis: Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics, version 20.0. Categorical variables were reported as frequencies and percentages, while time to positivity was presented as mean $\pm$ standard deviation or median with interquartile range according to distribution. The diagnostic sensitivity and negative predictive value of the five-day automated incubation protocol were calculated using combined automated detection and terminal subculture findings as the reference. A two-sided *p* value <0.05 was considered statistically significant.

Ethical considerations: The study was approved by the Institutional Ethics Committee. Patient confidentiality was maintained by analysing de-identified laboratory and clinical information.

RESULTS

Of the 1,878 aerobic blood-culture bottles evaluated, 287 (15.3%) were positive and 1,591 (84.7%) remained negative after five-day automated incubation. Positivity was comparable between adult and paediatric bottles (15.8% versus 13.1%; *p*=0.252), males and females (15.7% versus 14.8%; *p*=0.612), and different patient locations (*p*=0.328). Cultures collected after antimicrobial exposure had significantly lower positivity than pre-antimicrobial cultures (11.6% versus 18.6%; *p*<0.001). Central-line samples demonstrated greater positivity than peripheral samples (23.2% versus 14.4%; *p*=0.003). Loading delay beyond two hours was not significantly associated with automated positivity (*p*=0.180) [Table 1].

Table 1: Characteristics of aerobic blood-culture bottles and their association with automated culture positivity during five-day incubation (N=1,878).

Characteristic	Total	Automated positive	Automated negative	p value
	Frequency (%)			
Age category				
Adult	1,542 (82.1)	243 (15.8)	1,299 (84.2)	0.252
Paediatric	336 (17.9)	44 (13.1)	292 (86.9)	
Sex				
Male	1,031 (54.9)	162 (15.7)	869 (84.3)	0.612
Female	847 (45.1)	125 (14.8)	722 (85.2)	
Patient location				
Medicine wards	581 (30.9)	85 (14.6)	496 (85.4)	0.328
Intensive care units	413 (22.0)	75 (18.2)	338 (81.8)	
Emergency department	341 (18.2)	57 (16.7)	284 (83.3)	
Surgical wards	270 (14.4)	35 (13.0)	235 (87.0)	
Paediatrics/neonatology	209 (11.1)	27 (12.9)	182 (87.1)	
Other departments	64 (3.4)	8 (12.5)	56 (87.5)	
Antimicrobial therapy within preceding 48 hours				
Yes	891 (47.4)	103 (11.6)	788 (88.4)	<0.001
No	987 (52.6)	184 (18.6)	803 (81.4)	
Blood-collection site				
Peripheral venepuncture	1,697 (90.4)	245 (14.4)	1,452 (85.6)	0.003
Central venous catheter	181 (9.6)	42 (23.2)	139 (76.8)	
Time from collection to loading				
≤2 hours	1,604 (85.4)	253 (15.8)	1,351 (84.2)	0.18
>2 hours	274 (14.6)	34 (12.4)	240 (87.6)	
Overall	1,878 (100)	287 (15.3)	1,591 (84.7)	—

Among 287 automated-positive bottles, 180 (62.7%) flagged within 24 hours and 256 (89.2%) within 48 hours. Cumulative positivity reached 95.8% by 72 hours and 98.3% by 96 hours. Of the 245 clinically significant isolates, 171 (69.8%) were detected within 24 hours, 233 (95.1%) within 48 hours and

242 (98.8%) within 72 hours; all were detected by 96 hours. In contrast, all five bottles flagging between 96 and 120 hours yielded contaminants. The distribution of clinically significant isolates and contaminants differed significantly across incubation intervals ($p<0.001$) [Table 2].

Table 2: Distribution of automated blood-culture positivity according to incubation interval and clinical significance (n=287).

Incubation interval	All positive bottles	Cumulative positivity	Clinically significant isolates	Contaminants
	Frequency (%)			
≤24 hours	180 (62.7)	180 (62.7)	171 (95.0)	9 (5.0)
24.1–48 hours	76 (26.5)	256 (89.2)	62 (81.6)	14 (18.4)
48.1–72 hours	19 (6.6)	275 (95.8)	9 (47.4)	10 (52.6)
72.1–96 hours	7 (2.4)	282 (98.3)	3 (42.9)	4 (57.1)
96.1–120 hours	5 (1.7)	287 (100)	0	5 (100)
Total	287 (100)	—	245 (85.4)	42 (14.6)

Enterobacterales constituted the largest group of clinically significant isolates (116/245; 47.3%), followed by *Staphylococcus aureus* (39; 15.9%), non-fermenting Gram-negative bacilli (34; 13.9%) and *Candida* species (19; 7.8%). The overall median TTP for clinically significant organisms was 17.6 hours (IQR 12.4–25.9). Enterobacterales

demonstrated the shortest median TTP at 13.7 hours, whereas *Candida* species required 39.7 hours. TTP differed significantly among organism categories ($p<0.001$). Clinically significant organisms were detected substantially earlier than contaminants—17.6 versus 58.6 hours ($p<0.001$) [Table 3].

Table 3: Distribution and time to positivity of clinically significant organisms detected by the automated system (n=245).

Organism category	Principal organisms included	Frequency (%)	Time to positivity, hours median (IQR)
Enterobacterales	<i>Escherichia coli</i> (61), <i>Klebsiella pneumoniae</i> (42), <i>Salmonella</i> Typhi/Paratyphi (6), <i>Enterobacter</i> spp. (4), others (3)	116 (47.3)	13.7 (10.9–18.2)
Non-fermenting Gram-negative bacilli	<i>Acinetobacter baumannii</i> complex (18), <i>Pseudomonas aeruginosa</i> (16)	34 (13.9)	19.1 (14.6–26.8)
<i>Staphylococcus aureus</i>	Methicillin-sensitive (23), methicillin-resistant (16)	39 (15.9)	17.4 (13.2–23.9)
<i>Enterococcus</i> spp.	<i>E. faecalis</i> and <i>E. faecium</i>	17 (6.9)	20.8 (15.7–29.6)
Streptococci	β-haemolytic streptococci, viridans-group streptococci and <i>S. pneumoniae</i>	15 (6.1)	18.6 (13.9–27.4)
Other clinically significant Gram-positive cocci	Including <i>Aerococcus</i> and nutritionally variant streptococci	5 (2.0)	26.4 (20.1–38.7)
<i>Candida</i> spp.	<i>C. tropicalis</i> (7), <i>C. albicans</i> (5), <i>C. parapsilosis</i> (3), <i>C. glabrata</i> (3), other (1)	19 (7.8)	39.7 (28.8–57.2)
Total	—	245 (100)	17.6 (12.4–25.9)

TTP, time to positivity; IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*. Of the 1,591 bottles remaining automated-negative after five days, 1,584 (99.56%) showed no growth on terminal subculture. Seven bottles yielded growth, corresponding to an overall terminal-subculture recovery of 0.44% (95% CI 0.21–0.91). Only two

isolates—*Candida glabrata* and *Escherichia coli*—were considered clinically significant, giving an additional clinically significant yield of 0.13% (95% CI 0.03–0.46). The other five isolates were classified as contaminants. Terminal-subculture findings resulted in treatment modification in only one case, in which antifungal therapy was initiated [Table 4].

Table 4: Terminal-subculture findings among bottles remaining negative after five days of automated incubation (n=1,591).

Terminal subculture result	Automated-negative bottles Frequency (%)	Clinical classification	Effect on treatment
No microbial growth	1,584 (99.56)	True negative	None
<i>Candida glabrata</i>	1 (0.06)	Clinically significant	Antifungal treatment initiated
<i>Escherichia coli</i>	1 (0.06)	Clinically significant; concordant urine isolate	Existing antimicrobial therapy continued
Coagulase-negative staphylococci	3 (0.19)	Contaminants	No treatment modification
<i>Corynebacterium</i> species	1 (0.06)	Contaminant	No treatment modification
<i>Bacillus</i> species other than <i>B. anthracis</i>	1 (0.06)	Contaminant	No treatment modification
Any terminal-subculture growth	7 (0.44)	Significant: 2; contaminants: 5	Treatment modified in 1 case
Clinically significant terminal growth	2 (0.13)	False-negative automated cultures	—

The five-day automated protocol detected 245 of the 247 clinically significant isolates, while two were identified only through terminal subculture. The resulting sensitivity was 99.19% (95% CI 97.10–99.78), with an NPV of 99.87% (95% CI 99.54–99.97). Specificity was 97.42%, PPV was 85.37%, and overall diagnostic accuracy was 97.66%.

Agreement between the five-day automated result and the clinically adjudicated reference standard was almost perfect, with a Cohen's κ of 0.904. The comparatively lower PPV primarily reflected recovery of organisms subsequently classified as contaminants rather than analytical instrument errors [Table 5].

Table 5: Diagnostic performance of the five-day automated aerobic incubation protocol for clinically significant bloodstream isolates.

Automated five-day result	Clinically significant organism present	Clinically significant organism absent	Total
Positive	245	42	287
Negative	2	1,589	1,591
Total	247	1,631	1,878
Performance measure	Estimate, %	95% CI	
Sensitivity	99.19	97.10–99.78	
Specificity	97.42	96.54–98.09	
Positive predictive value	85.37	80.81–88.99	
Negative predictive value	99.87	99.54–99.97	
Overall diagnostic accuracy	97.66	96.87–98.25	
Cohen's kappa	0.904	—	

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Terminal-subculture growth was more frequent among bottles loaded more than two hours after collection than among those loaded within two hours (1.67% versus 0.22%; $p=0.012$). Growth was also numerically higher in bottles collected after antimicrobial exposure than in those collected before treatment (0.76% versus 0.12%), although the

difference did not reach statistical significance ($p=0.067$). Terminal recovery did not differ significantly between adult and paediatric bottles ($p=1.000$), peripheral and central-line samples ($p=0.119$), or intensive-care and other locations ($p=0.170$). These associations should be interpreted cautiously because only seven terminally positive bottles were identified [Table 6].

Table 6: Factors associated with terminal-subculture growth among automated-negative blood-culture bottles (n=1,591).

Factor	Automated-negative bottles	Terminal growth	No terminal growth	p value
	Frequency	Frequency (%)		
Antimicrobial therapy within preceding 48 hours				
Yes	788	6 (0.76)	782 (99.24)	0.067
No	803	1 (0.12)	802 (99.88)	
Time from collection to loading				
≤2 hours	1,351	3 (0.22)	1,348 (99.78)	0.012

>2 hours	240	4 (1.67)	236 (98.33)	
Age category				
Adult	1,299	6 (0.46)	1,293 (99.54)	1.000
Paediatric	292	1 (0.34)	291 (99.66)	
Collection site				
Peripheral venepuncture	1,452	5 (0.34)	1,447 (99.66)	0.119
Central venous catheter	139	2 (1.44)	137 (98.56)	
Patient location				
Intensive care unit	338	3 (0.89)	335 (99.11)	0.17
Other locations	1,253	4 (0.32)	1,249 (99.68)	

DISCUSSION

The present study demonstrated that five-day automated aerobic incubation provided highly reliable organism recovery. Of 1,878 bottles, 287 (15.3%) flagged positive, including 245 clinically significant isolates and 42 contaminants. Thus, the true-pathogen yield was 13.0%, while the overall contamination rate was 2.2%. These findings are comparable to the Indian study by Tarai et al., in which 13.7% of paired cultures were positive, comprising 10.45% true-positive cultures and 3.25% contaminants.^[14] The predominance of Enterobacterales, particularly *Escherichia coli* and *Klebsiella pneumoniae*, followed by *Staphylococcus aureus*, corresponds to the bloodstream-infection spectrum reported in Indian tertiary hospitals.^[14,15] However, incubation duration cannot compensate for insufficient blood volume or collection of only one bottle; Elantamilan et al. estimated that approximately 15% of positive episodes would have been missed if only a single specimen had been collected.^[15]

Most clinically significant organisms were detected rapidly: 95.1% were recovered within 48 hours, 98.8% within 72 hours and all automated clinically significant isolates within 96 hours. These findings closely resemble those of Tarai et al., who reported that 95.8% of isolates grew within 48 hours and 99.27% within 72 hours.^[14] Pardo et al. detected 98% of aerobic bacterial bloodstream infections by 48 hours and reported a 99.8% negative predictive value at that point.^[13] In the present analysis, pathogens had a substantially shorter median time to positivity than contaminants—17.6 versus 58.6 hours ($p<0.001$). This difference is biologically plausible because true bloodstream infection generally produces a larger viable inoculum, whereas contaminants are commonly introduced in small numbers during venepuncture. *Candida* species showed the longest median detection time at 39.7 hours, reflecting slower replication and lower metabolic carbon-dioxide production than most bacteria. Nevertheless, detection times are platform-dependent; Somily et al. found that BacT/ALERT VIRTUO detected 72.7% of tested organisms faster than BACTEC FX.^[16]

Only five bottles flagged positive between 96 and 120 hours, and all contained contaminants. Similarly, Péan de Ponfily et al. found that only 1% of 23,363 positive bottles flagged after four days, with very few newly identified clinically significant organisms [9]. AlMutawa and Delport reported that 99.2% of

positive cultures were detected within four days [10]. Earlier evaluations of Difco ESP and BACTEC 9240 indicated that shortening incubation to four days would miss only approximately 0.1% and 0.06% of significant isolates, respectively.^[11,12] Collectively, these comparisons suggest that four-day incubation may be operationally adequate for many contemporary systems. Nevertheless, platform-specific validation remains essential.

Among 1,591 bottles remaining negative after five days, terminal subculture yielded growth in seven (0.44%), but only two isolates (0.13%) were clinically significant. Consequently, the five-day protocol achieved 99.19% sensitivity, 99.87% negative predictive value, 97.66% accuracy and almost-perfect agreement with the adjudicated reference standard ($\kappa=0.904$). The positive predictive value of 85.37% should not be interpreted as an analytical false-positive rate because the instrument correctly detected microbial growth; rather, it reflects the clinical classification of 42 recovered organisms as contaminants. The very small additional yield and treatment modification in only one terminally detected case suggest that routine terminal subculture of every five-day-negative bottle would consume substantial resources for limited clinical benefit.

Pre-analytical factors remained important. Positivity was lower among patients already receiving antimicrobials than among untreated patients (11.6% versus 18.6%; $p<0.001$). Scheer et al. similarly reported positivity rates of 27.7% during antibiotic treatment versus 50.6% before treatment.^[17] while Cheng et al. observed a decline from 31.4% to 19.4% after antimicrobial administration.^[18] Although resin-containing media improve antibiotic neutralisation and organism recovery,^[21] they cannot completely reverse antimicrobial suppression. Delayed loading beyond two hours was also associated with terminal-subculture growth (1.67% versus 0.22%; $p=0.012$). Delayed entry may allow organisms to enter stationary phase before automated monitoring begins or may reduce the detectable metabolic signal. Deslandes et al. showed that pre-incubation delay prolonged total detection time.^[19] while Sautter et al. demonstrated increasing false-negative results with prolonged or inappropriate holding conditions.^[20] Overall, five-day incubation appears sufficient for routine aerobic cultures, provided that adequate blood volume, collection before antimicrobial administration, prompt loading and rigorous contamination control are maintained.

Limitations: This study was conducted at a single tertiary-care centre and evaluated only aerobic bottles, limiting generalisability to anaerobic, paediatric-specific, fungal and other automated culture protocols. The small number of terminal-subculture-positive bottles restricted meaningful multivariable analysis. Clinical classification of contaminants partly depended on available records and may be subjective. In addition, terminal subculture may introduce environmental contamination and cannot detect nonviable or non-culturable organisms.

CONCLUSION

The five-day automated aerobic blood-culture incubation protocol provided excellent recovery of clinically significant organisms, with 99.19% sensitivity and a 99.87% negative predictive value. Nearly all pathogens were detected within 72 hours, and none of the automatically detected clinically significant isolates appeared after 96 hours. Terminal subculture of five-day-negative bottles produced a very low clinically significant additional yield of 0.13%, suggesting limited value for its routine use. Nevertheless, recovery of two missed pathogens supports retaining the validated 120-hour protocol rather than universally shortening incubation. Optimal performance also requires adequate blood volume, collection before antimicrobial administration, prompt loading and strict aseptic technique to minimise contamination.

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