

Recovery of Bloodstream Pathogens using BD BACTEC and Brain Heart Infusion Broth in 350 Paired Samples in Western India

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Abstract:

Background: Blood culture remains central to the diagnosis of bloodstream infection. Automated systems provide continuous monitoring and early signalling of microbial growth, whereas conventional Brain Heart Infusion (BHI) broth depends on manual observation and subculture. This study also examined an experimentally important question: whether a BD BACTEC bottle can support microbial growth when incubated conventionally without instrument-based detection.

Objectives: To compare recovery of clinically significant bloodstream pathogens using BD BACTEC bottles and conventional BHI broth; to evaluate microbial growth in BD BACTEC bottles incubated without the automated instrument; and to distinguish the contribution of the bottle/medium from the practical advantage of automated detection.

Methods: A laboratory comparison conducted from April 2025 to April 2026 included 350 complete paired samples tested using automated BD BACTEC and conventional BHI. A manually incubated BD BACTEC bottle was also evaluated descriptively. Overall positivity was compared using the two-sided exact McNemar test. Organism recovery and four paired detection-time observations were summarized.

Results: BD BACTEC was positive in 106/350 samples (30.29%) and BHI in 39/350 (11.14%). Twenty samples were positive in both methods, 86 only in BD BACTEC, 19 only in BHI and 225 negative in both. The absolute positivity difference was 19.14 percentage points, with a crude positivity ratio of 2.72. Exact McNemar $p=2.30 \times 10^{-11}$. *Klebsiella* spp. recovery was 43 versus 18 and *E. coli* recovery was 32 versus 9. In four paired timing records, automated detection occurred 10–18 hours earlier than either manual method, averaging 13.5 hours earlier.

Conclusion: Automated BD BACTEC had significantly greater overall culture positivity than conventional BHI in the 350 paired samples. BHI nevertheless detected 19 samples that were negative in BD BACTEC. The manual BD BACTEC observation and four timing records remain descriptive and do not establish equivalence or a study-wide detection-time advantage.

Keywords: BD BACTEC; BHI Broth; Blood Culture; Manual Incubation; Bloodstream Infection; *Klebsiella*; *Escherichia Coli*; Neonatal Sepsis; Time To Positivity.

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Introduction

Bloodstream infections are associated with substantial morbidity and mortality, particularly in critically ill patients and neonates. Rapid microbiological diagnosis is important for targeted antimicrobial therapy and antimicrobial stewardship. Blood culture remains the reference

microbiological investigation for recovery of viable organisms from blood.

Blood-culture performance is influenced by blood volume, timing of collection, prior antimicrobial exposure, blood-to-broth ratio, culture-medium formulation, incubation conditions and the detection system. CLSI M47 emphasizes these critical factors

and includes specific guidance on pediatric blood cultures and selection of culture media.¹

Automated continuous-monitoring blood culture systems such as BD BACTEC combine specially formulated culture bottles with instrument-based monitoring. The instrument detects evidence of microbial growth and signals positivity, allowing earlier downstream Gram staining, subculture, identification and susceptibility testing. Conventional BHI broth is less technologically demanding but depends on manual observation and scheduled subculture.

A distinctive feature of the present investigation was an attempt to separate two components that are usually studied together: the growth-supporting capability of the culture bottle/medium and the additional contribution of automated detection. The study therefore evaluated BD BACTEC bottles both inside and outside the automated instrument and compared organism recovery with conventional BHI broth. Previous pediatric and resource-constrained laboratory comparisons provide context for this question.^{2,3}

Materials and Methods

Study Design and Setting: This laboratory-based paired comparison was conducted at NAMO Medical Education and Research Institute and Government Hospital, Silvassa, Western India, from April 2025 to April 2026. The primary analysis included 350 complete paired samples with automated BD BACTEC and conventional BHI results.

Three Arm Evaluation: 350 blood-culture sets were evaluated under three culture conditions: Arm A, a BD BACTEC bottle processed in the automated BD BACTEC instrument; Arm B, a second BD BACTEC bottle incubated conventionally without automated instrument-based detection; and Arm C, conventional BHI broth. The purpose was to determine whether organisms would grow in the BD BACTEC bottle when the automated detection component was removed and to observe the practical contribution of the instrument.

Microbiological Processing: BD BACTEC bottles were inoculated with 2 mL of blood. Incubation duration and the schedule of observation and subculture followed the laboratory blood-culture protocol. Cultures showing evidence of growth

underwent Gram staining and subculture onto appropriate solid media. Organisms were identified using standard microbiological procedures available in the laboratory. Antimicrobial susceptibility testing was performed for clinically significant isolates according to institutional procedures and applicable interpretive standards.

Outcomes and Statistical Approach: The primary outcome was sample-level culture positivity in 350 complete automated BD BACTEC–BHI pairs. Positivity percentages, the absolute paired positivity difference and the crude marginal positivity ratio were calculated. Organism-specific count ratios and the NICU proportion among 43 BD BACTEC *Klebsiella* isolates were secondary descriptive outcomes.

The two-sided exact McNemar test compared the discordant pairs: 86 BD BACTEC-positive/BHI-negative samples and 19 BD BACTEC-negative/BHI-positive samples. Under the null hypothesis, the discordant count follows a binomial distribution with $n=105$ and probability 0.5. Statistical significance was assessed at $p<0.05$. Neither culture method was treated as a diagnostic gold standard; sensitivity and specificity were not calculated.⁴

For a complete three-arm comparison, patient-linked outcomes from all three bottles would be required. No formal three-arm agreement or equivalence or inferential time-to-detection analysis was performed. Detection times were available for four samples across automated BD BACTEC, manually incubated BD BACTEC and conventional BHI, measured from the same starting point. Times and paired differences (manual minus automated detection time) were summarized descriptively. The timing subset selection criteria and exact starting event require documentation from the study records.

Results

Overall Paired Culture Positivity: BD BACTEC yielded 106/350 positive samples (30.29%), compared with 39/350 (11.14%) for BHI. The absolute positivity difference was 19.14 percentage points and the crude positivity ratio was 2.72. Both methods were positive in 20 samples; 86 were positive only in BD BACTEC and 19 only in BHI. Both were negative in 225. Overall agreement was 245/350 (70.00%). The two-sided exact McNemar p value was 2.30×10^{-11} ($p<0.001$).

Table 1. Paired culture results in 350 samples. The McNemar test uses the discordant cells 86 and 19.

BD BACTEC result	BHI positive	BHI negative	Total
Positive	20	86	106
Negative	19	225	244
Total	39	311	350

Recovery of predominant Enterobacterales: *Klebsiella* spp. and *E. coli* were the predominant organisms in the available comparison. *Klebsiella* recovery was 43 in the BD BACTEC dataset compared with 18 using conventional BHI, corresponding to a crude isolate-recovery ratio of 2.39. *E. coli* recovery was 32 versus 9, corresponding to a ratio of 3.56. Combined recovery of the two organisms was 75 versus 27, a crude ratio of 2.78.

Table 2: Organism-recovery counts and descriptive ratios. The combined *Klebsiella* and *E. coli* row is a subtotal and must not be added to the organism-specific rows. Organism-specific ratios are descriptive because organism-linked discordant cells are unavailable.

Organism	BD BACTEC	Conventional BHI	Crude recovery ratio
<i>Klebsiella</i> spp.	43	18	2.39
<i>Escherichia coli</i>	32	9	3.56
<i>Salmonella</i> spp.	1	1	1.00
Staphylococci/MRSA	22	10	2.20
Streptococci	8	1	8.00
Combined <i>Klebsiella</i> + <i>E. coli</i>	75	27	2.78

Salmonella spp. recovery was 1 in each method, staphylococci/MRSA recovery was 22 versus 10, and streptococci recovery was 8 versus 1. The five organism groups totalled 106 BD BACTEC and 39 BHI recoveries, consistent with the supplied positive-sample totals. The grouped staphylococci/MRSA category does not identify a separate MRSA count or contamination status.

NICU *Klebsiella* observation: Of the 43 *Klebsiella* isolates in the BD BACTEC dataset, 21 originated from NICU patients (48.8%) and 22 from other locations (51.2%). This finding is clinically relevant because neonatal blood cultures are particularly sensitive to preanalytical factors such as the limited blood volume available for inoculation.

Table 3: Location distribution of BD BACTEC *Klebsiella* isolates.

Source	<i>Klebsiella</i> isolates	Percentage
NICU	21	48.8%
Other locations	22	51.2%
Total	43	100%

Manual incubation observation: BD BACTEC bottles incubated manually without placement in the automated instrument were reported to support microbial growth. Manual incubation required observation and subculture rather than continuous instrument monitoring. Four samples had paired detection-time observations for all three methods, measured from the same starting point. Automated detection occurred 10–18 hours earlier than either manual method, with a mean paired difference of 13.5 hours. Manually incubated BD BACTEC and conventional BHI had identical recorded detection times in these four samples. The summaries do not establish equivalent recovery between automated and manually incubated BD BACTEC bottles.

Table 4: Characteristics of the three culture approaches.

Characteristic	Automated BD BACTEC	Manual BD BACTEC bottle	Conventional BHI
Supports microbial growth	Yes	Yes (observed experimentally)	Yes
Continuous automated monitoring	Yes	No	No
Automatic positive signal/alarm	Yes	No	No
Manual monitoring/subculture needed	After positive signal for work-up	Yes	Yes
Instrument based recognition of positivity	Yes	No	No
Dedicated detection instrument	Required	Not used experimentally	Not required

Table 5: Paired detection times in four samples, measured from the same starting point. Differences are manual detection time minus automated detection time.

Sample	Automated BD BACTEC (h)	Manual BD BACTEC (h)	Conventional BHI (h)	Automated earlier by (h)
A	12	24	24	12
B	18	36	36	18
C	20	30	30	10
D	14	28	28	14
Mean	16	29.5	29.5	13.5

Discussion

The primary finding was significantly higher overall positivity with automated BD BACTEC than with conventional BHI in 350 paired samples. The absolute difference was 19.14 percentage points. A second, descriptive observation was microbial growth in manually incubated BD BACTEC bottles.

The three-arm design attempts to distinguish the contribution of the bottle and medium from that of automated monitoring. Growth occurs within the medium, while the instrument monitors the bottle and signals evidence of growth. However, attribution of a recovery difference to medium formulation alone would require control of blood volume, antibiotic exposure and processing conditions. The four paired timing observations quantify earlier automated recognition in this subset. They cannot establish a study-wide time advantage, and scheduled manual observations may influence the recorded detection interval.

Published comparisons support the broader observation that automated BACTEC systems can improve or accelerate blood-culture detection compared with conventional approaches. Chokephaibulkit et al. compared an automated BACTEC system with a conventional system in hospitalized pediatric patients. Surase et al. compared BACTEC 9050 with conventional culture in a resource-constrained setting and reported higher overall culture positivity with BACTEC; their conventional protocol included BHI or Trypticase Soya Broth with scheduled subcultures.^{2,3}

The paired table provides the discordant results needed for McNemar testing. BD BACTEC detected 86 samples missed by BHI, whereas BHI detected 19 samples missed by BD BACTEC, giving 67 net additional positive samples with BD BACTEC. The exact paired test supports a difference in culture positivity under the study conditions. It does not identify either method as a gold standard or establish clinical sensitivity and specificity.⁴

The NICU *Klebsiella* observation is an important secondary finding rather than the primary proof of comparative performance. Nearly half of the *Klebsiella* isolates in the BD BACTEC dataset were from NICU patients. This supports further neonatal analysis because blood volume and other

preanalytical factors can substantially influence pathogen recovery.

The manual-incubation finding may be relevant to research on contingency approaches during instrument downtime or in resource-limited environments. It should not, however, be presented as evidence that manual incubation is equivalent to the manufacturer-recommended automated workflow. The observation is best described as proof-of-concept and should be prospectively validated before any routine clinical application.

Blood-culture comparisons should account for specimen collection, processing, reporting and clinical interpretation.⁵ Reviews of blood-culture optimisation also emphasise blood volume, sampling strategy and contamination control as determinants of recovery.⁶ The present organism counts therefore cannot isolate a medium effect from differences in collection or processing.

Blood volume is particularly important when interpreting the NICU subgroup. Inadequate volume has been associated with reduced detection of adult bacteremia,⁷ while experimental neonatal work has examined the relationship between inoculated volume, organism concentration and recovery.⁸ Low-level bacteremia has also been documented in children,⁹ and pediatric diagnostic reviews discuss volume and bottle selection.¹⁰ These studies provide context for the documented 2 mL BD BACTEC inoculation; they do not establish that this volume is optimal for every patient or that volumes were equivalent across all three arms.

The staphylococci/MRSA category requires clinical and microbiological clarification. Interpretation of a positive culture draws on organism identity, the pattern of positive sets and clinical findings when distinguishing infection from contamination.^{11,12} Adult data also show that the number of cultures obtained affects detection of bloodstream infection.¹³ The present grouped staphylococcal counts do not identify the number of MRSA isolates, contamination events or unique infection episodes.

Controlled comparisons of commercial blood-culture bottles illustrate the importance of evaluating specific bottle and medium combinations rather than treating all automated systems as interchangeable.¹⁴ Published incubation-duration

findings are likewise specific to the system, bottle and patient population evaluated.¹⁵ An incubation study using BacT/Alert FAN bottles cannot validate an incubation schedule for manually incubated BD BACTEC or conventional BHI. The exact local duration and subculture schedule must be reported from the study protocol, and study-wide assessment of detection speed requires complete paired timestamps beyond the four available samples.

Strengths and Novelty: The study combines a complete 350-sample paired automated BD BACTEC–BHI comparison with exploratory observations from manually incubated BD BACTEC bottles. The paired outcomes permit an appropriate within-sample positivity comparison. The NICU *Klebsiella* distribution provides a secondary clinical observation.

Recommended Prospective Validation: A future prospective paired protocol should inoculate equivalent, documented blood volumes from the same patient into three arms: automated BD BACTEC, manually incubated BD BACTEC, and conventional BHI. For every bottle, the study should record positive/negative status, organism recovered, exact time to automated positivity, exact time to manual detection, time to Gram stain and identification, contamination, prior antimicrobial exposure and all discordant results. This design would permit paired statistical testing and a direct estimate of the time advantage attributable to automated detection.

Conclusion

Among 350 complete paired samples, automated BD BACTEC was positive in 106 (30.29%) and conventional BHI in 39 (11.14%). The 86 versus 19 discordant pairs yielded a significant exact McNemar result ($p < 0.001$). BD BACTEC provided 67 net additional positive samples, although BHI uniquely detected 19 samples. Manually incubated BD BACTEC supported growth descriptively; four timing observations showed automated detection averaging 13.5 hours earlier. Complete manual-arm outcomes and timing data are needed to assess those secondary questions.

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