Biphasic versus monophasic manual blood culture bottles for low-resource settings: an in-vitro study

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Summary

Background Manual blood culture bottles (BCBs) are frequently used in low-resource settings. There are few BCB performance evaluations, especially evaluations comparing them with automated systems. We evaluated two manual BCBs (Bi-State BCB and BacT/ALERT BCB) and compared their yield and time to growth detection with those of automated BacT/ALERT system.

Methods BCBs were spiked in triplicate with 177 clinical isolates representing pathogens common in low-resource settings (19 bacterial and one yeast species) in adult and paediatric volumes, resulting in 1056 spiked BCBs per BCB system. Growth in manual BCBs was evaluated daily by visually inspecting the broth, agar slant, and, for BacT/ALERT BCB, colour change of the growth indicator. The primary outcomes were BCB yield (proportion of spiked BCB showing growth) and time to detection (proportion of positive BCB with growth detected on day 1 of incubation). 95% CI for yield and growth on day 1 were calculated using bootstrap method for clustered data using. Secondary outcomes were time to colony for all BCBs (defined as number of days between incubation and colony growth sufficient to use for further testing) and difference between time to detection in broth and on agar slant for the Bi-State BCBs.

Findings Overall yield was 95·9% (95% CI 93·9–98·0) for Bi-State BCB and 95·5% (93·3–97·8) for manual BacT/ALERT, versus 96·1% (94·0–98·1) for the automated BacT/ALERT system (p=0·61). Day 1 growth was present in 920 (90·8%) of 1013 positive Bi-State BCB and 757 (75·0%) of 1009 positive manual BacT/ALERT BCB, versus 1008 (99·3%) of 1015 automated bottles. On day 2, detection rates were 100% for BI-State BCB, 97·7% for manual BacT/ALERT BCB, and 100% for automated bottles. For Bi-State BCB, growth mostly occurred simultaneously in broth and slant (81·7%). Sufficient colony growth on the slant to perform further tests was present in only 44·1% of biphasic bottles on day 2 and 59·0% on day 3.

Interpretation The yield of manual BCB was comparable with the automated system, suggesting that manual blood culture systems are an acceptable alternative to automated systems in low-resource settings. Bi-State BCB outperformed manual BacT/ALERT bottles, but the agar slant did not allow earlier detection nor earlier colony growth. Time to detection for manual blood culture systems still lags that of automated systems, and research into innovative and affordable methods of growth detection in manual BCBs is encouraged.

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Introduction

Blood culture is essential for clinical management of bloodstream infections, microbiological surveillance, and antibiotic stewardship. Historically, it was conducted using manual blood culture bottles (BCBs) that were visually inspected for signs of growth (eg, haemolysis, turbidity, puffballs, and gas production).[1] However, in the 1990s, automated growth detection systems were developed using the continuous measurement of carbon dioxide production. Nowadays, these automated alternatives are standard in high-resource settings and are widely considered to be the best available blood culture method. However, automated systems remain poorly suited to laboratories in settings with basic laboratory and health-care resources (henceforth referred to as low-resource settings) where high costs and environmental factors (eg, heat, humidity, power fluctuations, and dust) complicate their use.[2] Current forecasting estimates that use of manual BCB will continue to increase and will account for two-thirds of the market share by 2025.[3] Yet, despite their prevalence, research on their performance has been nearly non-existent since the advent of automated systems and it is unclear whether low-resource settings are using substandard approaches to blood culture.

Budgetary, logistic, and infrastructure challenges restrict the use of blood culture in low-resource settings,[2,4] yet the global rise in antimicrobial resistance demands diagnostic and clinical management tools to be available in all settings.[5] In 2016, Médecins Sans Frontières launched a project to develop clinical bacteriology laboratories adapted to low-resource settings (known as the Mini-Lab).[6] Easy growth detection in manual BCBs is key to our evaluation,

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Research in context

Evidence before this study

Although non-automated, manual blood culture bottles (BCBs) make up most of the global blood culture market, research evaluating such bottles has not been done since the advent of automated systems in the 1990s. Manual biphasic BCB, which contain both a broth (liquid phase) and an agar slant (solid phase), provide useful tools to simplify manual blood culture in settings with limited laboratory and health-care resources (ie, low-resource settings). We searched PubMed for original studies, published from database inception to Oct 1 2020, using the terms "biphasic", "agar slide", "agar slant" AND "blood culture", restricted to English. Studies on mycobacteria or specific pathogens were excluded. We identified 13 studies that compared the ability of biphasic bottles to detect bacterial or yeast infections with other BCB types, and none of those studies compared biphasic BCB with current automated systems, which are the standard in high-resource settings. The available, albeit scant, evidence showed suitable and rapid recovery of Gram-positive bacteria and yeasts in biphasic bottles, compared with other BCB types.

Added value of this study

In high-resource settings, manual BCBs have been replaced by automated systems, which means there are few studies from these countries that evaluate how their systems compare with older technology. Clinicians and researchers in low-resource settings, where manual bottles are still widely used, might be too under-resourced or understaffed to assess the performance of their culture equipment. This study is the first to provide an in-vitro comparison between biphasic, manual, and automated BCBs. We used whole blood spiked with 177 strains of pathogenic bloodstream microorganisms, with a focus on strains commonly found in low-resource settings, to simulate adult and paediatric volumes, microbial concentrations, and growth characteristics and assess whether the most commonly used global technique for blood culture (manual BCBs) was substandard. Our findings suggest that the yield of manual BCBs is comparable with that of automated blood culture systems, but speed of growth lags, and that the time to actionable results for manual BCBs could be shortened considerably if blind subculture was systematically performed, regardless of visual signs of growth, after overnight incubation. Biphasic BCBs allowed faster growth detection than the monophasic manual BCB we tested, but the added value of the agar slant for colony recovery was small. Moreover, this study describes the most common signs of growth for the different manual bottle types, which could be useful for laboratories in low-resource settings that interpret and identify pathogens.

Implications of all the available evidence

In settings where automated blood culture systems are unavailable, manual BCBs are an acceptable alternative. These results show that performing an early subculture can speed the recovery of colonies for further testing. By showing that the added value of the agar slant was low, our findings highlight how further research into biphasic BCB design is needed to allow colony recovery from agar slants.

so we selected biphasic bottles (ie, BCBs combining a broth with an agar slant) for this study; these bottles were chosen by the Mini-Lab team in collaboration with the Mini-Lab Scientific Committee. This BCB type was initially developed in the 1940s to detect *Brucella* species, which require long incubation times.[7] Colonies growing on the agar slant enable growth evaluation and allow technicians to bypass subculture to identify bacteria and conduct antibiotic susceptibility testing, shortening the time to colony by 18–24 h. Published studies on the performance of biphasic BCBs is scarce and are from the 1970s and 1980s,[8–20] but generally these bottles are associated with better Gram-positive species recovery and faster bacterial growth than monophasic BCBs (ie, broth-based BCBs without agar), although growth detection on agar slant is variable (appendix pp 13–14).[8–11,15,16,18–20]

See Online for appendix

We aimed to evaluate two manual BCBs and compare them with an automated blood culture system for detection of common bloodstream pathogens.

Methods

Study design and cultures

Following a 2018 market review and BCB target product profile, we identified three biphasic BCB brands for potential evaluation (appendix pp 2–9). The two best-scoring BCBs, Bi-State BCBs (Autobio Diagnostics, Zhenzhou, China) and manual BacT/ALERT BCBs (bioMérieux, Marcy-l'Etoile, France), were selected for this study (appendix p 15) and compared with the automated BacT/ALERT blood culture system (bioMérieux) at the Institute of Tropical Medicine (Antwerp, Belgium).

For simulated paediatric blood cultures (different volumes and bacterial concentrations), we used Bi-State bottles and BacT/ALERT PF Plus bottles. For simulated adult blood cultures, we used Bi-State bottles and the BacT/ALERT FA Plus bottles. BacT/ALERT PF Plus bottles and BacT/ALERT FA Plus bottles have identical content but are marketed by the manufacturer as paediatric (for BacT/ALERT PF Plus bottles) and aerobic adult bottles (for BacT/ALERT FA Plus bottles). The barcode on the paediatric bottles triggers a different reading algorithm.

Leftover blood from blood bank volunteers of Red Cross Flanders (Mechelen, Belgium) was used under a research contract (file number CM20180327A). Clinical strains were obtained from the Institute of Tropical Medicine travel clinic and microbiological surveillance studies.[21–24] An Institutional Review Board of the

	Number of strains tested in paediatric patients	Number of strains tested in adult patients	Proportion of strains tested in human blood	Microbial group	Geographical origin
Acinetobacter baumannii	3	3	3/6 (50%)	Non-fermenters	Democratic Republic of the Congo; Ecuador
Burkholderia cepacia	21	21	30/42 (71%)	Non-fermenters	Benin; Democratic Republic of the Congo; Ecuador
Candida tropicalis	2	2	0	Yeast	Burkina Faso; Democratic Republic of the Congo
Citrobacter freundii	3	3	3/6 (50%)	Enterobacterales	Cambodia; Democratic Republic of the Congo
Enterobacter cloacae	3	2	3/5 (60%)	Enterobacterales	Benin; Cambodia; Ecuador
Escherichia coli	19	20	29/39 (74%)	Enterobacterales	Benin; Burkina Faso; Cambodia; Democratic Republic of the Congo; Ecuador
Enterococcus faecalis	3	3	3/6 (50%)	*Staphylococcus*; *Enterococcus*	Benin; Democratic Republic of the Congo
Haemophilus influenzae	20	20	0	Fastidious	Belgium; Burkina Faso; France
Klebsiella oxytoca	3	3	0	Enterobacterales	Benin; Ecuador
Klebsiella pneumoniae	20	20	0	Enterobacterales	Benin; Burkina Faso; Cambodia; Democratic Republic of the Congo
Neisseria meningitidis	3	3	0	Fastidious	Burkina Faso
Pseudomonas aeruginosa	3	3	3/6 (50%)	Non-fermenters	Benin; Cambodia; Democratic Republic of the Congo
Staphylococcus aureus	20	20	30/40 (75%)	*Staphylococcus*; *Enterococcus*	Benin; Burkina Faso; Cambodia, Democratic Republic of the Congo
Stenotrophomonas maltophilia	3	3	6/6 (100%)	Non-fermenters	Cambodia; Democratic Republic of the Congo
Streptococcus pneumoniae	19	19	25/38 (66%)	*Streptococcus*	Burkina Faso; Cambodia; Democratic Republic of the Congo
Streptococcus pyogenes	3	3	6/6 (100%)	*Streptococcus*	Burkina Faso; Cambodia; Democratic Republic of the Congo
Streptococcus suis	3	3	6/6 (100%)	*Streptococcus*	Cambodia
Salmonella enterica serotype Typhi	3	3	6/6 (100%)	Enterobacterales	Cambodia; Democratic Republic of the Congo; Peru
Salmonella enterica serotype Typhimurium	20	20	29/40 (73%)	Enterobacterales	Benin; Burkina Faso; Cambodia; Democratic Republic of the Congo; The Gambia
Streptococcus agalactiae	3	1	3/4 (75%)	*Streptococcus*	Cambodia
Total	177	175	135/352 (52%)	..	..

Data are n or n/N (%).

Table 1: **List of bacterial and yeast strains used with their geographical origins**

University of Antwerp (reference 613/08) and the Ethical Committee of the University Hospital of Antwerp (reference 8/20/96) approved the surveillance studies and the use of bacterial isolates for future studies involving diagnostics.

Blood spiking and BCB inoculation

Blood was spiked using frozen (–80°C) reference strains and anonymised clinical strains from pathogens that frequently cause bloodstream infections in low-resource settings (table 1).[25,26] 162 (91·5%) of 177 strains originated from patients infected in low-resource settings (obtained through blood culture surveillance studies in low-resource settings or via the Institute of Tropical Medicine travel clinic). The strains originating from Europe (15 [8·5%] of 177; all *Haemophilus influenzae*) were obtained either from the travel clinic or as part of external quality assessment programmes in Belgium (reference strains). 175 strains, corresponding to 20 different microbial species, were inoculated in all bottle types. Paediatric bottles were inoculated with three extra strains; adult bottles were inoculated with one extra strain as well (*Escherichia coli*; logistic and time constraints of the Mini-Lab project precluded inoculation in adult bottles of these strains).

Human banked blood from volunteers was used from Nov 6, 2018, until April 9, 2019. In May, 2019, a change in sampling procedure at the transfusion blood bank led to the unavailability of whole human blood. Defibrinated horse blood (E&O Laboratories, Bonnybridge, UK) was used for the remainder of the experiments (1512 [47·7%] of 3168 bottles) after determining equivalence in yield and speed of growth (appendix pp 10–12). Table 1 shows the percentage of microbial strains tested in human blood.

Dilution series were prepared using a 0·5 McFarland suspension in sterile saline (figure 1). Of the final suspension (estimated concentration of 375 colony-forming units [CFU] per mL), 100 µL was plated out in triplicate on blood agar plates; the mean of the colony counts was used to calculate the number of CFU added to the BCBs. The final suspension was added to blood and

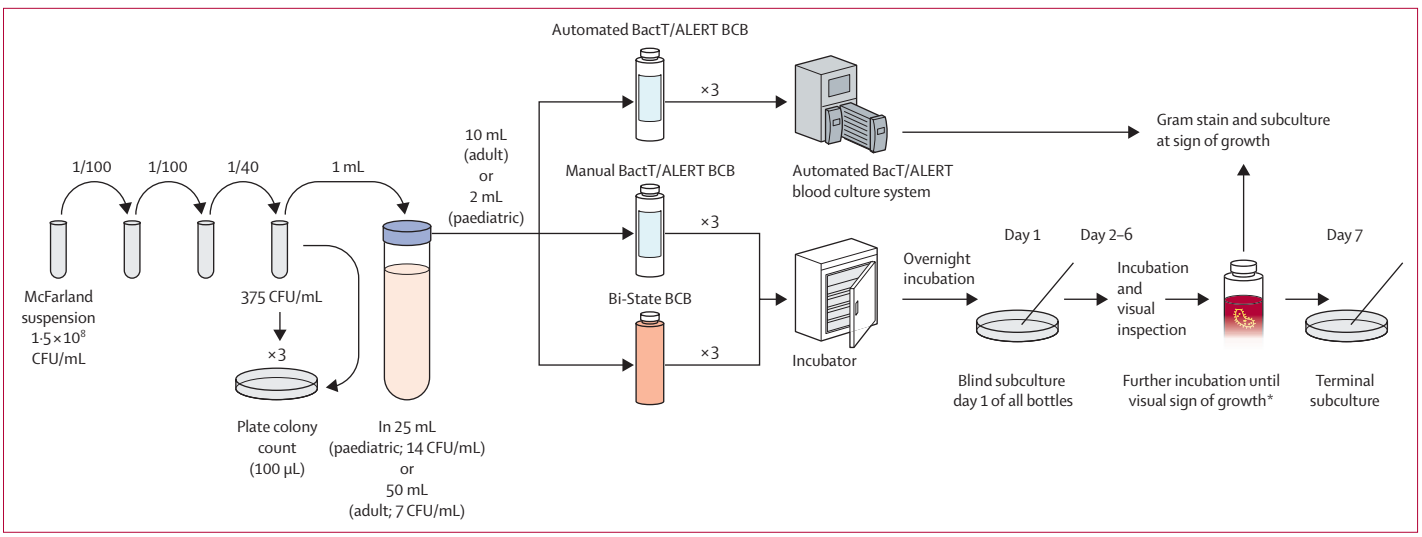

Figure 1: **Blood spiking and inoculation of the blood culture bottles**
BCB=blood culture bottle. CFU=colony-forming units. *Turbidity, haemolysis, bacterial puffballs, gas production, and colony growth on slant (for Bi-State BCB) or chromogenic indicator colour change (for BacT/ALERT BCB).

inoculated in the paediatric and adult BCBs, resulting in an estimated end concentration of 14 CFU/mL of blood for paediatric (2 mL; blood-broth ratio 1:15) and 7 CFU/mL for adult (10 mL; blood-broth ratio 1:3) experiments.

For *Candida tropicalis*, we omitted one dilution step (1:100), as 0·5 McFarland suspension in yeasts corresponds to just 1–5×10⁶ CFU/mL.[27] In total, 1056 bottles were inoculated per blood culture system (ie, Bi-State, manual BacT/ALERT, and automated BacT/ALERT). A mean of three 100 µL colony counts of bacterial and yeast suspension was used to calculate the number of CFU added to the BCBs. When inoculating BCBs with spiked blood, one bottle of each BCB type was also inoculated with non-spiked blood as a sterility control.

BCB processing

Manual BCBs were incubated in a static incubator at 35°C and inspected for signs of growth twice daily for the first 2 days and once daily from day 3 onward. Turbidity, haemolysis, bacterial puffballs, gas production, and colony growth on slant (for Bi-State BCBs) or chromogenic indicator colour change (for BacT/ALERT BCBs) were assessed by the same laboratory technicians who inoculated the BCBs (unblinded). Date and time of first appearance of growth signs in the broth (all BCBs), change in chromogenic indicator (for manual BacT/ALERT BCBs), and growth signs on the agar slant (for Bi-State BCBs) were recorded. BCBs were incubated for 7 days. Biphasic BCB agar was flooded at inoculation by laying the bottle horizontally for 15 min and again after 48 h of incubation if no colony growth was present at that time. A lightbox (JP Selecta, Barcelona, Spain) providing diffuse light was used for daily visual inspection of the BCBs. A blind subculture (regardless of visual growth signs in the bottle) on chocolate agar (blood agar number 2 base; Becton Dickinson; Franklin Lakes, NJ, USA) was performed for all manual BCBs after one night of incubation (day 1 subculture). A second subculture on chocolate agar was performed if the blind subculture was negative and visual growth signs in the bottle appeared after day 1 of incubation. A Gram stain was performed at visual growth detection.

Automated BacT/ALERT BCBs were placed in the BacT/ALERT 3D 120 (bioMérieux) blood culture system, which assures incubation at 35°C, continuous agitation, and frequent (every 10 min) automated growth detection by measuring colour changes of the chromogenic indicators in the BCBs. A chocolate agar subculture and Gram stain were performed at the moment of growth detection. Day 1 blind subculture was not performed for the automated BCBs as it is not advised to remove these bottles from the incubator to assure continuous temperatures.

If no growth occurred on any subculture by day 7, a terminal subculture on chocolate agar was performed for all BCBs.

Pathogen grouping

For some analyses, pathogens were grouped according to common growth and clinical characteristics into Enterobacterales; glucose-non-fermenting Gram-negative bacteria (henceforth referred to as non-fermenters); *Streptococcus*, *Staphylococcus* or *Enterococcus* species; fastidious organisms (ie, *Neisseria meningitidis* and *Haemophilus influenzae*); and yeast species (table 1).

Outcomes

Primary outcomes were BCB yield and time to detection. Yield was defined as proportion of spiked BCBs that turned positive. Positivity of BCBs was defined as colony

	Total tested per bottle type	Median number of CFU added to bottles (IQR)	Bi-State BCBs (95% CI)	BacT/ALERT manual BCBs (95% CI)	BacT/ALERT automated system (95% CI)
Adult volume	525	34 (18–56)	98·1% (96·1–100·0)	97·5% (95·5–99·6)	97·9% (95·9–99·9)
Paediatric volume	531	13 (7–25)	93·8% (90·5–97·0)	93·6% (90·2–97·0)	94·4% (91·3–97·5)
Total	1056	20 (10–42)	95·9% (93·9–98·0)	95·5% (93·3–97·8)	96·1% (94·0–98·1)

BCB=blood culture bottle. CFU=colony-forming unit.

Table 2: **Yield of spiked bottles with confirmed growth in adult and paediatric experiments**

growth of the inoculated organism on any subculture or microscopic visualisation of bacteria concordant with the inoculated strain on Gram stain at visual or automate growth detection, in the absence of growth on subculture. Yield was further stratified by bacterial group to detect suboptimal performance by species (defined as yield <90%). Contamination was defined as colony growth or microscopic morphology of bacteria not concordant with the inoculated strain.

For manual BCBs, time to detection was expressed as the proportion of positive bottles showing visual growth signs on day 1 of incubation. For automated BCBs, a time to detection, as registered by the instrument, of less than 28 h was considered as growth on day 1, assuming laboratory working hours from 0800 h to 1700 h. All visual signs of growth were documented with the day and time of their first occurrence.

Secondary outcomes were time to colony (ie, the time between incubation and the availability of accessible colonies for further testing) for all BCBs and difference between time to detection in broth and on agar slant for the Bi-State BCBs. Time to colony was defined as the number of days between incubation and the appearance of accessible colonies, either on agar slant or subculture, for further testing. Accessible colonies on the agar slant were defined as sufficient growth on agar slant for further testing, consisting of large single colonies or a thick confluent layer of growth.

Ease of use assessment

The laboratory technicians involved in the study (n=3) were surveyed about the bottles' ease of use, septum thickness, ability to detect growth, and access to colonies on agar. The survey was done at the end of the laboratory experiment period in March, 2020.

Statistical analysis

A priori sample size calculation was not performed before the start of the study. Data was collected on worksheets and entered into Microsoft Excel 2019 (version 2110). Statistical analyses were done in R using RStudio (version 4.0.2).

95% CI for yield and growth on day 1 were calculated using bootstrap method for clustered data using the aods3 R package (version 0.4–1.1). Independent proportions (eg, contamination across bottle types) were compared using χ^2 tests. Random effects logistic regression models were used to model associations, expressed as odds ratios (ORs), between BCB variables (BCB type, adult *vs* pediatric BCB, and number of CFU added to the BCB) and yield while allowing for clustering by bacterial strain (lme4 package [version 1·1–23]). Similar regression models were used to assess the association of BCB type and growth on blind subculture, the association between BCB variables and growth on day 1 among positive bottles, and the association between BCB type and yield for species with suboptimal performance. Threshold for significance was determined at p<0·05.

Role of the funding source

The funder of the study was involved in the study design, data interpretation, and writing of the manuscript. However, an independent scientific committee was installed to safeguard scientific integrity of the study.

Results

Final median plate colony count was 16 CFU (IQR 9–29), lower than expected (based on dilution calculations of 37–38 CFU). Plate counts ranged from 0–162 CFU, resulting in a median bacterial spiked blood concentration of 6 CFU/mL (IQR 3–12; range 0–40) for paediatric experiments and 3 CFU/mL (IQR 1–6; range 0–32) for adult experiments.

Yield for the different bottle types is shown in table 2. Overall yield was 96·1% (1011 of 1052 bottles) for the BacT/ALERT automated system, 95·9% (1009 of 1052) for Bi-State BCBs, and 95·5% (1005 of 1052) for manual BacT/ALERT BCBs. Paediatric bottles showed lower odds of growth than adult bottles (OR 0·10, 95% CI 0·05–0·18, p<0·0001), but the difference was no longer significant when corrected for number of CFU added (0·64, 0·27–1·54; appendix p 16). Yield was less than 90% for *Burkholderia cepacia* and *Neisseria meningitidis* only, and there were no significant differences between BCB types in yield for these species (Bi-State *vs* automated BacT/ALERT p=0·65; Bi-State *vs* manual BacT/ALERT p=0·49; appendix p 17). Contamination occurred in 27 (0·9%) of 3168 BCBs in similar proportions across the bottle types (p=0·89).

Overall median time to detection for automated BCBs was 13·6 h (IQR 12·3–15·8). The longest median time to detection was observed in non-fermenters at 19·9 h (17·4–21·3; appendix p 21).

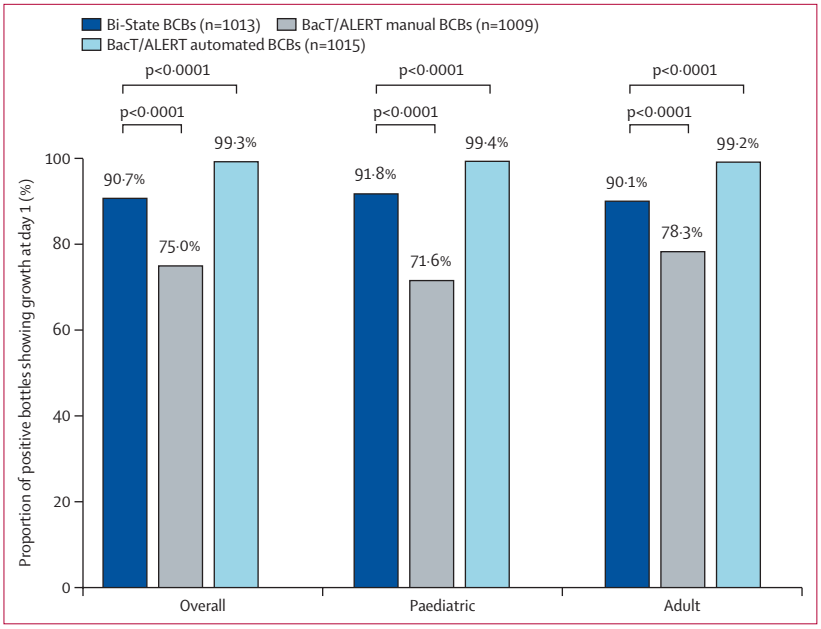

Figure 2: **Proportion of positive bottles showing growth at day 1 for the different blood culture bottles**
Fastest growth was observed for the automated BacT/ALERT bottles, both paediatric and adult formulations.

Growth detection was faster in the automated system than manual systems, with time to detection less than 28 h (ie, day 1 growth) for 1008 (99·3%) of 1015 automated bottles (95% CI 98·5–100; figure 2). For positive Bi-State bottles, day 1 growth was 90·8% (920 of 1013 bottles; 87·0–94·5), although only 75·0% (757 of 1009; 68·8–81·2) for manual BacT/ALERT bottles (p<0·0001; figure 2). After two nights of incubation, all of the positive automated and Bi-State bottles and 986 (97·7%) of 1009 positive manual BacT/ALERT bottles showed growth. In manual BCBs, non-fermenters and fastidious organisms showed the slowest growth. For non-fermenters, growth detection on day 1 was observed in 57 (36·8%) of 155 positive Bi-State bottles and 34 (21·7%) of 157 positive manual BacT/ALERT bottles. For fastidious organisms, growth detection on day 1 was observed for 120 (88·9%) of 135 positive Bi-State bottles and 24 (17·8%) of 135 manual BacT/ALERT BCB (appendix p 18).

For Bi-State BCBs, visual growth signs appeared simultaneously in broth and on agar slant in 828 (81·7%) of 1013 positive bottles. Broth growth preceded visible agar colonies in 169 (16·7%) of 1013 positive bottles, whereas agar growth preceded growth in broth in only 16 (1·6%) of 1013 positive bottles.

There was no significant difference in colony growth after day 1 blind subculture of Bi-State versus BacT/ALERT BCBs (OR 0·40, 95% CI 0·10–1·65, p=0·20; appendix p 19). Time to colony was significantly shortened by performing systematic day 1 blind subculture; because of routine day 1 blind subculture, colonies were available for 1010 (99·7%) of 1013 of Bi-State BCBs and 1001 (99·2%) of 1009 of manual BacT/ALERT BCBs on day 2. If subculture been performed only at the moment of visual growth signs as opposed to blind subculture, colonies would have been available on day 2 for 920 (90·8%) of 1013 of Bi-State bottles (p<0·0001) and 757 (75·0%) of 1009 of manual BacT/ALERT bottles (p<0·0001).

For biphasic bottles, agar slant growth did not shorten time to colony because accessible colonies were tardive (appendix p 20). Only 447 (44·1%) of 1013 of positive biphasic bottles had sufficient colony growth (enough to pick colonies from the slant) on day 2 of incubation, slower than subculturing BCBs with visible growth (920 [90·8%] of 1013 colonies on subculture by day 2) or day 1 blind subculture (1010 [99·7%] of 1013 colonies by day 2).

Visual signs of growth varied by bottle type and inoculated organism (appendix p 22). For both manual bottle types, turbidity was the fastest growth sign (821 [81·0%] of 1013 Bi-State BCBs *vs* 827 [82·0%] of 1009 for BacT/ALERT BCBs). Chromogenic indicator colour change was the most common growth sign for BacT/ALERT BCBs (present in 1000 [99·1%] of 1009 BacT/ALERT BCBs), but was only seen as first signs of growth in 803 (79·6%) of 1009 BacT/ALERT BCBs.

Visual growth signs in Bi-State bottle broth and on agar slant by bacterial group are shown in table 3. Turbidity was the most common sign of growth for all bacterial groups except species of the *Staphylococcus* or *Enterococcus* genera, where a white film on red blood cells and confluent growth on agar were more commonly detected. Gas production was a common sign of Enterobacterales (388 [92·6%] of 419 Bi-State BCBs), but not for species belonging to the other microbial groups (47 [7·9%] of 594). Bacterial puffballs were more frequently observed for *Staphylococcus* or *Enterococcus* species (101 [73·2%] of 138) than for other species (83 [9·5%] of 875). Sufficient growth on agar slant for further testing was present in 65·1% of positive Bi-State bottles by the end of the incubation period (table 3; confluent growth [thick layer] and single colonies [large] were counted as sufficient growth).

According to the three surveyed laboratory technicians involved in the study, shelf life of Bi-State bottles (1·5 years) was better suited to low-resource settings than was that of BacT/ALERT bottles (1 year), as was the ease of assessing visual growth and labelling bottles (BacT/ALERT's opaque labels were reported to impede easy interpretation of the culture). BacT/ALERT bottle material (autoclavable polycarbonate) and filling indicators were considered easier to fill, interpret, and use. The very thick septum of Bi-State bottles made BCBs inoculation more difficult than for BacT/ALERT bottles. A detailed ease-of-use comparison can be found in the appendix (p 21).

Discussion

Our results show that, in simulated laboratory conditions, biphasic BCBs and automated blood culture system yields were similar, and that growth could be detected very early (within 28 h) in manual bottles (although still

somewhat delayed compared with automated blood cultures). Up to 90·7% of biphasic BCB growth was detected visually on the first day of incubation (versus 99·3% in automated bottles), although manual BCB day 1 blind subculture resulted in pickable agar slant colonies on day 2 for almost all Bi-State bottles. When automated bottles were subcultured once flagged positive (assuming 0800–1700 h laboratory working hours), they yielded a similar proportion (99·3%) of colony growth on day 2. Therefore, performing day 1 blind subculture of manual BCBs led to comparable time to colony for manual and automated BCBs. Since the timing of pathogen identification and antibiotic susceptibility testing depends on when these colonies are available for testing, an automated system would not have led to actionable results any earlier than a manual system that includes day 1 blind subculture.

The biphasic bottles produced growth significantly earlier than manual BacT/ALERT bottles evaluated in this study, leading to faster visual detection nearly 15% of the time. Day 1 blind subculture, however, had similar outcomes for both bottle types, suggesting that BacT/ALERT's slower growth could be due to the slower appearance of visual signs of growth. BacT/ALERT bottles are also not designed for visual growth detection (although this strategy has been used in low-resource settings),[24,28] and have large labels that impede broth inspection. However, even if not ideal, our results also confirm that these bottles designed for automated incubators can also be used manually if needed (eg, in case of technical problems with the automatic instrument). Bi-State bottles' agar slant allows easy observation of colony growth. The considerable difference in time to detection between BacT/ALERT bottles incubated in automated versus static incubators might be due to agitation in the automated incubator (known to speed growth in some organisms, particularly strictly aerobic ones).[29] Less sensitive growth detection by visual inspection compared with automated evaluation of these bottles could also contribute to this difference.

The biphasic bottle agar had little added value (<2% of growth was detected earlier through detection of agar slant colonies). In only 65·1% of biphasic BCBs, colonies on the slant were accessible for further testing by the end of the incubation period, precluding reliance on the bottle slant to replace subculture on solid agar (table 3). Bi-State bottles were considered easy to use, except for septum rigidity, which could increase the risk of needle stick injury. An additional concern is that the bottle material is not suitable for autoclaving (Bi-State BCBs are made of polyethylene terephthalate), which deformed heavily when autoclaved with liquid and agar spill. Reuse of the BCBs by autoclavation, which is a common practice in low-resource settings, is thus not possible.

Our findings differ to previous studies. The timing of accessible agar slant growth was mostly too late to be of clinical value, diverging from previous research in which at least 48% of isolates had earlier accessible agar slant

	N	Signs of growth in broth						Signs of growth on agar slant				
		Turbidity	Gas production	Bacterial puff balls	White film on red blood cells	Pellicle on broth surface	Haemolysis (%)	Bubbles in agar (%)	Confluent growth, thin film (%)	Confluent growth, thick layer (%)	Single colonies, small (%)	Single colonies, large (%)
Enterobacterales	419	410 (97·8%)	388 (92·6%)	9 (2·1%)	359 (85·7%)	0	60 (14·3%)	388 (92·6%)	167 (39·9%)	227 (54·2%)	18 (4·3%)	26 (6·2%)
Non-fermenters	155	152 (98·1%)	7 (4·5%)	33 (21·3%)	135 (87·1%)	0	1 (0·6%)	6 (3·9%)	31 (20·0%)	97 (62·6%)	7 (4·5%)	19 (12·3%)
Staphylococcus or *Enterococcus*	138	113 (81·9%)	8 (5·8%)	101 (73·2%)	123 (89·1%)	7 (5·1%)	73 (52·9%)	6 (4·3%)	3 (2·2%)	131 (94·9%)	3 (2·2%)	3 (2·2%)
Streptococcus	154	141 (91·6%)	26 (16·9%)	23 (14·9%)	125 (81·2%)	9 (5·8%)	137 (89·0%)	16 (10·3%)	40 (26·0%)	74 (48·1%)	31 (20·1%)	20 (13·0%)
Fastidious organisms*	135	132 (97·8%)	6 (4·4%)	18 (13·3%)	129 (95·6%)	0	0	6 (4·4%)	15 (11·1%)	111 (82·2%)	3 (2·2%)	9 (6·7%)
Yeast	12	12 (100%)	0	0	6 (50·0%)	0	0	0	12 (100%)	0	0	5 (41·7%)
Total	1013	960 (95·7%)	435 (42·9%)	184 (18·2%)	577 (86·6%)	16 (1·6%)	217 (26·8%)	422 (41·7%)	268 (25·0%)	640 (57·5%)	62 (6·7%)	82 (7·6%)

Data are n or n (%). **Haemophilus influenzae* and *Neisseria meningitidis*.

Table 3: **Visual signs of growth in broth and on agar slant for positive Bi-State bottles by bacterial species**

growth than subculture.[15,18] These earlier results could be partly related to a BCB design that physically separated the agar slant from broth.[9,18] This bottle design was possibly less affected by humidity and constant submergence in the broth, which washes away the colonies, but is no longer commercially available. Other biphasic designs, such as the slide blood culture system marketed as Septi-Chek (Becton Dickinson), show similar promising clinical result but are also no longer commercially available. These bottle designs might have become obsolete in settings that use automated systems but could still be of important use for blood culture systems in low-resource settings.

Flooding time and frequency was another notable difference between our study and most published studies on biphasic bottles. In other studies,[8,9,10–18,20] agar slants or paddles were flooded daily with the broth, usually for only a few seconds. We followed Hemoline (bioMérieux) bottle instructions for use, flooding the agar for 15 min after inoculation and repeating the flooding after 48 h if no colonies were visible at that time.

Our results should be interpreted and extrapolated with care because of the distinct differences that existed between our study environment and an active clinical laboratory. Day 1 blind subculture yield and growth speed were higher than clinical conditions, with studies reporting a 82–85% pathogen recovery rate using day 1 blind subculture, including in low-resource settings.[30,31] This finding could be because we had higher spiked blood bacterial concentrations than what is typically observed in septic patients in health-care settings (<10 CFU/mL)[1]. Moreover, our growth detection was conducted by expert, non-blinded laboratory technicians, so even subtle signs were likely to be interpreted as signs of growth. Additionally, in a clinical laboratory, most BCBs will be negative, which elevates the threshold of declaring a bottle as positive. In such a setting, agar slant growth would be a more straightforward sign to interpret as positive. Another discrepancy from real-life conditions is the immediate incubation of bottles after inoculation. Longer needle-to-incubator times, expected in clinical settings, could alter yield and time to detection. The clinical relevance of our results is also affected by laboratory working hours and the effect of Gram stain on clinical decision making. With standard laboratory working hours (0800–1700 h) and blind subculture, the time to colony is comparable for manual and automated systems since no investigations will be done overnight. However, in a 24 h functioning laboratory, automated systems have a comparative advantage because of continuous growth monitoring. Gram stain results are available sooner with automated systems. In our study, time to detection and time to colony for manual BCBs were highly reliant on blind subculture. The benefits of faster results must, however, be balanced against an increased contamination risk when performing blind subculture in a real-life diagnostic setting. This risk is not negligible and could substantially increase the financial costs of blood cultures and unnecessary treatment, especially in low-resource settings.[1] Moreover, blind subculture implies higher costs (because of consumables and staff). Optimal timing of blind subculture can also differ according to setting.[31]

Additionally, plasma substances in septic patients (including antibiotics) inhibit bacterial growth. Patients in low-resource settings might be on empiric antibiotic therapy before they reach the hospital, which can affect BCB yield and growth speed.[32] BacT/ALERT bottles contain resins that absorb antibiotics and could show better performance in clinical studies than those without resin in settings where pre-hospital antibiotic consumption is high.[33] Finally, because of unforeseen unavailability of human blood during our study, we had to switch to horse blood for a large proportion of the BCBs (1512 [47·7%] of 3168 BCBs). This limitation did not affect comparison of the BCB types because all BCB types were inoculated in parallel, but could have affected the generalisability of the results to clinical settings.

Current biphasic bottle designs might not be worth further investment because the slant was found to be of little value. However, a different biphasic BCB design could make a substantial difference and should be the subject of future research. Having less of the agar slant submerged in broth improves access to colonies. Larger bottles can accommodate a higher broth volume, optimising the blood–broth ratio. Given the prospects of an increasing market for manual BCBs in low-resource settings,[3] research and development into new (or revived) bottle types should be encouraged. Similarly, optimised BCB autoclaving procedures should be researched. Scientific guidance on this topic is currently absent and BCB materials (eg, polyethylene terephthalate) are often not compatible with autoclavation. Research on the optimal composition and quality assessment of homemade manual BCBs could also be important for future implementation of blood cultures in low-resource settings. Although not within the scope of this study, our research group is currently evaluating optimal composition of homemade BCBs to accommodate this widespread practice.

In this study, biphasic BCBs had a similar yield as automated BCBs and detected growth faster than manual BacT/ALERT bottles. Agar slant growth, however, was inconsistent, species-dependent, and often too tardive to bypass the need for subculture. Time to detection was shorter for automated BCBs than for manual BCBs, but differences in time to colony were small, especially when day 1 blind subculture was performed. These results suggest that commercially available manual BCBs are sufficiently sensitive to be used in low-resource settings that cannot afford automated blood culture systems. We did not observe an added value of the agar slant of biphasic BCBs, so monophasic manual BCBs could have similar performance. Research into other biphasic BCB designs, other manual BCBs, and the optimal composition of homemade BCBs is needed.

Contributors

SO, AN, J-BR, OV, JJ, and LH conceptualised the study. SO, JJ, and LH designed the study. SO acquired the data and did the data analysis. SO, TK, AN, LH, JBR, JJ, and OV interpreted and presented the data. SO, JJ, and LH drafted the manuscript. AN, J-BR, TK, and OV revised the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. SO and LH accessed and verified the data.

Declaration of interests

TK reports travel fees from Médecins Sans Frontières, during the conduct of the study. All other authors declare no competing interests.

Data sharing

The dataset is available from figshare. The laboratory procedures are available from protocols.io.

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For the **study dataset** see https://doi.org/10.6084/m9.figshare.14994057.v1

For the **study procedures** see https://www.protocols.io/view/validation-of-manual-blood-culture-bottles-bsqqndvw