Opinion Paper

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Collection, transport and storage procedures for blood culture specimens in adult patients: recommendations from a board of Italian experts

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Abstract: Bloodstream infections (BSIs) remain a potentially life-threatening condition. The gold standard for the diagnosis of BSI is still blood cultures (BCs), and the diagnostic yield depends on clinical and technical factors that have an impact on collection and transportation. Hence, monitoring of the entire pre-analytical process from blood collection to transportation to the microbiology laboratory is critical. To optimize the clinical impact of the diagnostic and therapeutic procedures, a multidisciplinary approach and univocal protocols are mandatory. A board of specialists discussed the available evidence on the pre-analytical process and produced the present document to guide physicians and nurses on the ideal execution of BC: (1) timing and preparation for blood collection; (2) skin antisepsis; (3) blood volume; (4) sampling method and safety; (5) medium to be used; (6) time to BC transportation; and (7) quality assurance and quality management.

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Introduction

Bloodstream infection (BSI) is a potentially life-threatening condition with a case fatality rate of 30%–40% and increasing incidence and severity [1, 2]. The rapid and accurate diagnosis of bacteremia and fungemia allows prompt administration of targeted antibiotic therapy (within 24–48 h), with positive effects on the clinical outcomes, reducing mortality and costs, and limiting the development of antimicrobial resistance [3].

The diagnosis of BSI relies on the detection of pathogens, bacteria or fungi in blood cultures (BCs), still considered as the gold standard to identify pathogens and assess their susceptibility profile. The appropriate collection and quick transportation of BCs strongly influence the diagnostic yield. Moreover, safety during BC sampling needs to be tackled using the correct procedures by engaging specialized and well-trained staff [4–6].

The aim of this article is to provide a practical guide for physicians and nurses on the optimal execution of BC, as recommended by a board of specialists after in-depth discussion of the available evidence in this field. The document contains practical recommendations on the clinical and technical operational procedures of the entire BC pre-analytical process: (1) timing and preparation for blood collection; (2) skin antisepsis; (3) blood volume; (4) sampling method and staff safety; (5) medium to be used; (6) time to BC transportation; and (7) quality assurance and quality management.

Timing and preparation for blood collection

The most appropriate timing for BC collection is the first aspect that needs to be carefully considered.

Unfortunately, only few clinical studies evaluating the timing of BC collection are available. Although all guidelines agree that samples for BC should be collected before antimicrobial treatment, it remains controversial whether they should be drawn at or around the time of a fever spike, as physicians often prescribe. In a multicenter retrospective study evaluating the timing of BC collection in relation to temperature increases in patients with BSI, it was found that the likelihood of diagnosing BSI was not significantly enhanced if BC collection was done when patients experienced temperature spikes [7]. Lamy et al. [8] evaluated several studies and concluded that there is no evidence that clearly identifies any optimal timing for BC collection.

- Therefore, BC samples should be collected as soon as possible when BSI is suspected and before antibiotic use.
- BC sampling must be performed by specialized and well-trained staff with specific expertise in the various phases of the procedure. Ideally, dedicated phlebotomy teams should be created [9] and this may also be cost-effective [10–12]. However, several organizational and economic limitations, along with cultural habits, hamper their implementation in many countries.
- The entire procedure must be carefully standardized and registered. Every health care facility should define and develop core elements and complete a checklist of items related to the pre-analytical steps. Collection of all BC samples must be clearly documented in the patient's medical records, indicating the date and time of blood collection, skin preparation, sampling site and strategy used [13].
- Blood collection should be carefully organized by preparing and checking all devices and materials needed for the draw, including safety butterfly sets and personal protective equipment as needed.
- To reduce the risk of BC contamination at the time of blood collection, hand hygiene must be carefully performed using a hydroalcoholic preparation, and disposable gloves of the correct size must be worn [14]. Wearing disposable gloves is not an alternative to hand hygiene, which must be carried out again after removing the gloves [15]. The use of sterile gloves is not strictly necessary, although it could be considered depending on the institution's contamination rate [16]. Even after good hand hygiene and wearing gloves, the vein must not be re-palpated after skin antisepsis and before puncture [17].
- As a general rule, peripheral venipuncture is the method of choice for BC collection. A suitable peripheral venous site must be identified before skin antisepsis.

In the absence of a suitable peripheral venous site, an arterial blood site can be considered a valid alternative, because arterial blood has been shown to perform similar to venous blood in terms of contamination and sensitivity [18]. Sampling through pre-existing intravenous catheters, port-a-cath or central venous catheters increases the risk of contamination [18–21], and the microbiological results have to be interpreted cautiously. However, there are clinical situations in which access to a peripheral vein may be difficult; this may be particularly evident in critically ill and often edematous patients in the intensive care unit (ICU), for whom peripheral phlebotomy is challenging [22–24].

Although discouraged, this method can be accepted when venous catheter sampling is performed from a freshly placed cannula [17]. One exception is the diagnosis of central line-associated BSI; most current guidelines recommend simultaneous sampling of two BC sets, one collected from the catheter and the other through a, possibly contralateral, venipuncture to be able to estimate the differential time to positive BC or different microbial load [25–27].

Skin antisepsis

Antisepsis of the skin must be performed rigorously at the time of peripheral venous sampling to avoid specimen contamination and consequent alteration of the test results.

A BC contamination rate of 3% is generally considered the maximum acceptable value [28]. The microbiology laboratory of every health care facility should analyze the data on contamination of BCs at set time intervals (e.g. every 6 months) and discuss them with the clinical team if necessary.

- As indicated by a multidisciplinary team review of best practices for BC collection, skin antisepsis must be performed using 2% chlorhexidine in 70% isopropyl alcohol [21], preferably using disposable devices registered as a medicine. The pre-selected site should be disinfected by scrubbing an area of skin 6–7 cm in diameter for 30 s and then waiting about 30 s for the antiseptic to dry. Cleaning the skin treated with chlorhexidine after blood withdrawal is not necessary.

If the patient is allergic to chlorhexidine, alcoholic povidone-iodine can be used for 120 s [21].

When blood collection through a venous, peripheral or a central catheter is unavoidable, great attention must also be paid to disinfect the connection between the catheter and the drawing set (i.e. the needleless connector or the injection port) using a specific product for the appropriate time: 2% chlorhexidine in 70% isopropyl alcohol for a minimum of 30 s and allowed to dry before using the system [14].

The use of sterile disposable devices seems to have various advantages in terms of standardization, exact dosage, sterility, mechanical action and effectiveness [29].

– The stopper of each BC bottle is not sterile and must be disinfected before inoculation. A specific antiseptic can be used to disinfect the stopper, similar to that used for the skin (2% chlorhexidine in 70% isopropyl alcohol) or 70% isopropyl alcohol alone.

Blood volume

An adequate volume of blood is the most important parameter for the detection of microorganisms in the bloodstream of patients with suspected BSI, because the bacterial and fungal load in most BSIs is very low [8, 30–33].

– Recommendations for complete BC sampling suggest at least two sets, corresponding to 30–40 mL in total [24], subdivided into four BC bottles, two for aerobic and two for anaerobic organisms; the aerobic bottle should be filled first. BC should take priority in the case of several specimens from the same venipuncture [26, 28, 34–40].
– Recently, a retrospective study of 4000 BSI episodes demonstrated that without a third BC set, 7.9% of BSIs would have been missed, although in 33.9% of the missed episodes, the same organism recovered in the third BC was isolated in other clinical specimens (i.e. urine) for an actual rate of approximately 5% [39].
– The practice of a "solitary BC" (only two bottles or one set), although still common, is not acceptable and should be actively discouraged [8, 28, 35].
– BC bottles should be inoculated with 8–10 mL of blood (according to the manufacturer's instructions). When BC bottles are underfilled (i.e. less than 5 mL of blood per bottle), false-negative results or delays in microbial growth may occur [8, 22]. When BC bottles are overfilled (>10 mL), they are at increased risk of being flagged as false-positive mainly as a result of high production of background CO_2 by the white blood cells [41, 42].

The correct filling of BC bottles should be monitored through visual inspection, either at the patient's bedside or on arrival at the microbiology laboratory before BC incubation [8, 43]. Weighing the BC bottle when delivered to the laboratory is an alternative strategy [8]. Indeed, some BC system manufacturers have developed automated systems to estimate the level in the bottle at the time of loading [44].

– It has been reported that one aerobic bottle plus one anaerobic bottle yield more pathogens than two aerobic bottles, confirming that an anaerobic bottle should be included in each BC set [45].

An adequate volume of blood can be obtained either by increasing the number of venipunctures ("multi-sampling strategy") or by collecting an adequate volume through one single venipuncture ("single-sampling strategy"), with comparable performance and similar sensitivity for a given blood volume inoculated [8]. The multi-sampling strategy is the standard approach recommended for decades, based on the execution of at least two separate BC sets within a short period (generally 10–12 min apart). The single-sampling strategy, introduced more recently, is based on the total volume of blood collected from one single draw and inoculating the requested BC bottles (four to six in total) at the same time [8, 46, 47]. Both approaches have advantages and limitations. In particular, the single-sampling strategy has important advantages: it allows a sufficient volume of blood to be collected from a single sample with simultaneous filling of at least four bottles; reducing the contamination rate by limiting the number of blood draws; reducing the workload and therefore the risk of occupational exposure to pathogens; reducing the rate of solitary BCs; and enabling early initiation of empirical antibiotic treatment without waiting for subsequent BC sampling [8, 21, 23, 38, 46, 48–51].

In a multi-sampling strategy, a positive result depends on the number of positive BC samples found when a culture yields a common contaminant such as coagulase-negative staphylococci. In this case, the result is clinically significant only if there are at least two positive BCs [8]. With the single-sampling strategy, the rules for the interpretation of positive BC results are different, and the probability of having a clinically significant result increases with the number of positive bottles [8].

In general, the single-sampling strategy seems to be preferable in some types of patients, such as those in the ICU and emergency department. Conversely, the multi-sampling strategy is required to diagnose infective endocarditis, for which the number of positive samples on

three separate venipunctures over 24 h is part of the modified Duke Criteria [52].

Sampling method and safety

Needlestick injuries represent a significant proportion of the work-related accidents recorded in hospitals; therefore, equipment and techniques for BC sampling must guarantee the operator's safety [4, 5]. In accordance with international recommendations and the European Directive 2010/32/EU [6], devices must be adopted that guarantee the safety of the patient and the health operator: a sampling set with a butterfly needle equipped with a safety mechanism and an adapter for the collection of multiple blood specimens directly into the vials. The use of the same syringe to draw blood and then to inoculate the bottles must be avoided. The practice of capping sharp objects has been banned by the Directive 2010/32/EU. At the end of blood withdrawal, the safety mechanism should be activated and the sampling set disposed of in the appropriate container for sharp waste.

Medium to be used

In addition to the recommendation to always use both aerobic and anaerobic bottles, it remains optional to use a third bottle specifically for fungal detection and bottles with special media (i.e. enriched with substances that improve microbial recovery by absorbing antimicrobial agents present in the blood or that lyse the white blood cells releasing the microorganisms).

In patients with candidemia, the sensitivity for detecting *Candida* growth varies depending on the species, the system and the type of vials used [53]; sensitivity rates are lower in patients with neutropenia and those undergoing antifungal treatment [54]. The quantitative burden of *Candida* is very low in most cases; more than 50% of *Candida* BCs have ≤1 CFU/mL [33]. Similar to bacteria, a BC collection of four to six bottles is recommended, and, following these recommendations, a sensitivity of approximately 50%–75% has been reported to detect *Candida* in BCs.

Most clinical laboratories do not routinely use mycosis media for all BCs because most cases of candidemia can be successfully diagnosed using aerobic blood vials [55–57]; the exception is some *Candida* spp., such as *C. glabrata*, which is particularly prone to growing more frequently or more quickly in anaerobic vials [54, 56, 58, 59]. Therefore, for some *Candida* spp. and depending on the system and type of bottles used, mycosis bottles need to be included [54, 60]. Yeasts other than *Candida* in BCs have been increasingly reported as emerging and rare pathogens in up to 5% of patients with fungemia, and lysis/centrifugation methods are more suitable for detecting these rare yeast pathogens in BCs with higher efficacy [54].

- Although BCs must be collected before starting antibiotic treatment, in some situations this is not possible, especially in those cases where symptoms of bacteremia persist due to inappropriate antimicrobial treatment [37].
- The superior neutralizing capacity of resin-based media has been demonstrated, although different resins may affect the performance of neutralization [61, 62].

A valid alternative to the use of media containing resins is the dilution of blood in broth at ratios greater than 1:5, which increases microbial recovery, probably by diluting antimicrobial agents and natural inhibitory factors in the blood to subinhibitory concentrations [63].

Time to BC transportation

The transport time of inoculated BC bottles to the laboratory and their incubation in continuous monitoring systems is a critical factor, and any delay beyond the maximum limit can postpone or hamper microbial growth [64, 65].

- The inoculated BC bottles must be sent as soon as possible to the microbiology laboratory.
- A time of 2 h between sample collection and incubation in a BC system can be considered optimal [24, 28]. The maximum acceptable limit is 4 h; beyond this time, a delay might negatively affect the positive BC rate and the time to prompt and effective antimicrobial therapy [64, 66–69]. Although the optimal time to transportation should be <2 h, even a threshold of 4 h may be difficult to achieve in many situations [67, 70].

To follow these recommendations, health care facilities must implement adequate organization to make rapid delivery of BC bottles to a microbiology unit possible. Therefore, it is not acceptable to accumulate the BC samples inoculated throughout the day and send them together to the laboratory. Inoculated BC bottles, if not delivered to the laboratory immediately, must be stored at room temperature, avoiding temperatures higher than 30 °C or refrigerated conditions [24].

– In health care organizations where immediate transport of BCs to the laboratory for incubation in BC continuous monitoring systems cannot be guaranteed, and in those institutions where the microbiology service is not active 24 h per day, 7 days per week, the use of satellite BC instruments for incubation in specific settings, such as emergency departments and the ICU, has been suggested to allow significant improvements in terms of time and quality of the results [71]. However, the level of evidence remains limited, and further studies are needed to assess costs and benefits associated with such an organization.

Good practices for execution of BC are presented in Table 1.

Table 1: Good practice for the execution of blood cultures.

– Perform hand hygiene
– Collect all the necessary materials and place them on a clean tray (checking the expiration dates of all the items to be used)
– Mark the optimal filling volume on the vials
– Wear gloves
– Apply the tourniquet
– Perform antisepsis of the pre-selected site for sampling with 2% chlorhexidine in 70% isopropyl alcohol (disposable sterile applicator) for 30 s
– Allow to dry for 30 s
– Do not palpate the vein again (if the maneuver is necessary, wear sterile gloves)
– Remove the stopper from the vial for blood culture and disinfect (2% chlorhexidine in 70% isopropyl alcohol)
– Allow to dry for 30 s
– Withdraw blood using a safety butterfly needle with an adapter for the collection of blood directly into the vials
– A complete blood culture sampling comprises at least 4–6 vials (2–3 sets)
– Keep the vial upright under the patient's arm
– Fill every vial with approximately 10 mL of blood (adult patients), filling the aerobic vial first and then the anaerobic vial
– Remove the tourniquet as soon as the blood begins to flow or within 2 min from application
– Remove the vials gradually as they are filled and shake them gently
– Activate the safety system of the needle used on removal from the vein
– Dispose of the needle in the appropriate rigid container for sharp waste
– Carry out hemostasis with a dry swab
– Remove the gloves and perform hand hygiene
– Mark all the necessary information on the vials (number of the sample, sampling site, time and date)
– Indicate execution of blood culture sampling in the patient's medical record
– Send the vials to the laboratory immediately
– If available, immediately place the vials into the satellite incubators

Modified from [13].

Quality assurance and quality management

Quality control of the pre-analytical phase should be monitored regularly by critical key performance indicators; several have been proposed and are in use [70]. The first step is to clearly communicate to the clinician and all the staff involved uniform and standardized guidelines for correct BC collection. Indicators allow different parts of the pre-analytical phase of BCs to be monitored. Monitoring the total volume of blood collected using systematic internal programs is a strong quality assurance requirement [8, 22, 24, 44, 70–73]; the proportion of solitary BCs, which should be as low as possible (<10%), should be monitored.

False-positive and false-negative results are also essential indicators of analytical processes [8, 69, 70]. Moreover, the contamination rate should be assessed and should be less than 3% [21, 24, 74–78]. Although no definitive criteria are available, distinguishing contaminated BCs from those that are true-positive cultures is possible primarily by identifying the organism responsible for bacteremia and the number of positive BC samples or bottles with the same bacterial species. Moreover, clinical and laboratory information, the possible infection source, and assessment of the time to positivity may represent additional markers useful in clinical settings to discriminate between contamination and true-positive BCs [24, 74]. Contamination rates can be diminished by revised procedures and techniques, such as skin antisepsis, optimum sampling site and a single-sampling strategy [8, 24]. Monitoring the percentage of bottles collected via an indwelling intravascular access device instead of venipuncture BC collection and those obtained percutaneously is also important [77].

The time to transportation to the laboratory is another important key indicator. Because earlier detection is achieved if the transport time is minimized, it has been proposed that the proportion of BCs submitted with a delay should be monitored [76]. Although the critical limit that should not be exceeded has not yet been defined, it is advisable to have the shortest time delay possible [70].

A list of recommendations and comments on the correct procedures and techniques for BC collection are given in Table 2.

Discussion and conclusions

To ensure rapid and accurate BC results, it is mandatory to adequately select, collect and transport the specimens,

Table 2: Recommendations for the correct procedures and techniques for collecting blood cultures.

Pre-analytical phase	Recommendations	Comments
BC timing and preparation	Collect BCs when BSI is suspected before administration of antibiotics	There is no need to wait for temperature spikes or chills. Have BCs collected by a specialized or well-trained team
	Hand hygiene must be carefully performed with a hydroalcoholic preparation followed by wearing disposable gloves of the correct size	Both hand hygiene and wearing gloves are necessary. It is not necessary to wear sterile gloves
	Peripheral venipuncture is the method of choice for BC collection. Alternatively, sampling by the arterial blood site could be considered, with similar performance	Sampling from an arterial blood site performs better than from pre-existing intravenous catheters; the latter are associated with higher rates of contamination. Sampling via catheters is necessary for the diagnosis of central line-associated BSI
Skin antisepsis	Use 2% chlorhexidine in 70% isopropyl alcohol for 30 s; in allergic patients, use alcoholic povidone-iodine for 120 s	Rates of contamination should be as low as possible (desirable ≤3%). The use of sterile disposable devices is recommended
Blood volume	The blood volume to be collected for each set[a] must be at least 20–30 mL; minimum number of sets: 2; optimal number of sets: 3; minimum volume of blood, 30–40 mL, equal to 4 bottles (2 sets); optimal volume of blood: 40–60 mL, equal to 6 bottles (3 sets); volume per bottle: 8–10 mL	Small total volume or underfilled bottles may be associated with false-negative results or delays in microbial growth; overfilled bottles could have a negative impact on the diagnostic yield of BC, with false-positive results (due to high leukocyte counts) or false-negative results (caused by blood clots); "solitary" BCs (only 2 bottles or 1 set) may negatively affect the sensitivity of BC and are not acceptable
Sampling method and safety	Use of a butterfly needle equipped with a safety mechanism and an adapter for the collection of multiple blood specimens directly into the vials	It is acceptable to use either a vacutainer or needle and syringe; the double-needle technique is not recommended
	Single-sampling strategies (SSS) and multi-sampling strategies are used. SSS is preferred	SSS can help reduce the risk of contamination and has the advantage of requiring only one venipuncture
BC media	The use of both aerobic and anaerobic bottles with resin-based media is preferred when antibiotic administration has started before the collection of BCs	Most cases of candidemia can be successfully diagnosed using aerobic blood vials; *C. glabrata* grows better in anaerobic bottles
	In some circumstances, mycosis bottles need to be included	For some *Candida* spp. and depending on the system and type of bottles used, mycosis vials are needed
Time to BC transportation	Optimal transport time to incubation: 2 h; store BC samples at room temperature before incubation	The critical maximum time limit has not yet been defined, but it should be as short as possible; a delay might negatively affect the diagnostic yield of BC; use satellite BC instruments for incubation in some situations
Indicators for quality assurance and quality management	Total volume of blood cultured: the volume of blood per bottle and the number of bottles	Assess the proportion of solitary BCs, the number of false-positive and false-negative results, and check the bottle weight and/or the fluid level in the bottles manually or assisted by instruments
	Contamination rate	As contamination has a negative impact on BC specificity, it should be as low as possible (≤3%); check procedures, such as skin antisepsis, sampling site and sampling strategy
	Time to transportation	Monitoring the proportion of BCs submitted with a delay (optimal time: ≤2 h; maximum: ≤4 h)

BC, blood culture; BSI, bloodstream infection. [a]One set consists of 1 aerobic plus 1 anaerobic bottle.

a process that includes several steps from sample withdrawal to incubation [8, 24, 48, 69, 78–80].

The process involves various health personnel, who must operate in agreement with good clinical practice within a multidisciplinary team. In addition to the doctors and nurses engaged in the diagnostic process, the hospital pharmacist with infectious disease training plays an important role, because he/she is responsible for the procurement, proper preservation and, together with the other personnel involved, the choice of pharmaceutical

products required for correct BC practice, which undeniably have an impact on the validity of the test [69, 81–85].

Despite the existence of some national and international reviews on the correct procedures and techniques for the pre-analytical phase, adopting the current recommendations is a critical issue in many cases [8, 24, 28, 70]. In many hospital wards, the execution of BCs is insufficient in numerical terms and often incorrect, especially within emergency departments where limited time and the large number of patients requiring critical care often result in incorrect procedures [74]. Although most cases of BSI come from the community, making the involvement of emergency departments essential, shortcomings of a cultural (often there is a delay in transferring the patient to the department after initiating empirical antibiotic treatment) and organizational nature (lack of microbiology laboratories open 24 h a day or lack of satellite incubators that enable sampling to be performed at any time) make the execution of BC tests suboptimal.

Hence, organizational and cultural operative interventions must be planned and implemented in clinical practice. In particular, with regard to cultural and educational aspects, certain potentially effective actions are advisable:

1. Dissemination of protocols and their application: for the first time, this document brings together the operative procedures that constitute the BC test;
2. Interventions on communication and training of medical and nursing personnel (drawing up and disseminating authoritative documents, courses, but also practical training at the patient's bedside, bringing together all the various professional figures involved: doctor, nurses, microbiologist and others);
3. The formation, within the hospital, of a BC team comprising nurses, clinical microbiologists and infectious disease experts, who have expertise in sampling and, as a second step, the establishment and review of the treatments involving other professional figures (intensive care experts, clinicians, hospital pharmacists, etc.).

To translate these actions into practice, it is vital to increase awareness among Health Management and Scientific Societies that have the task of organizing training courses and educational initiatives, as well as providing the theoretical and organizational framework required for optimal clinical practice [86]. The medico-legal and health cost aspects associated with the accuracy and reliability of BC tests should also be underlined. The incorrect execution of a BC can, for example, lead to legal disputes and claims for compensation. Moreover, a false-positive result, with the consequent increase in antibiotic treatments and hospital stays, can translate into higher health costs, but also in a pointless and damaging selective pressure on which antimicrobial resistance emergencies are based. Finally, extending awareness of the problem of BSI is to be hoped for beyond the health care field, among institutions, the media and the general population. It is known that 80% of septic events arise in communities [87]; increasing awareness and educating people to recognize the initial symptoms will reduce fatal outcomes due to late interventions.

BSI is a global concern that needs to be adequately considered and tackled with a multidisciplinary approach. Although other recommendations have been released for the correct management of the pre-analytical phase, including venous blood sampling and sample transportation, BC specimens require more detailed and specific guidelines [88–90].

To guarantee the clinical effectiveness of BC and the best possible patient outcome, it is paramount that the involvement of the various professionals is based on constant communication and strict adherence to shared procedures. These aspects of communication and adherence to univocal protocols are currently the main issues that must be addressed to optimize the clinical impact of the diagnostic and therapeutic procedures. Only in this way will it be possible to reduce the rate of morbidity and mortality that BSIs still cause worldwide.

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