Italian Consensus Document

Execution, transport and preservation procedures for sampling for blood culture in cases of suspected sepsis

With the endorsement of

AMCLI associazione microbiologi clinici italiani

SOCIETÀ ITALIANA DI FARMACIA OSPEDALIERA E DEI SERVIZI FARMACEUTICI DELLE AZIENDE SANITARIE

SIM SOCIETÀ ITALIANA DI MICROBIOLOGIA

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Italian Consensus Document

Execution, transport and preservation procedures for sampling for blood culture in cases of suspected sepsis

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Published with the unconditional contribution
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Printed in xxxxxxxxxx

Scientific board

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1. INTRODUCTION

Blood culture is the primary test for the diagnosis of infections of the bloodstream and an essential component of the clinical management of a patient with sepsis (defined as an organ dysfunction hazardous to life caused by an altered response to infection [1]). The isolation of bacteria or fungi from the blood has an important diagnostic (as confirmation of the general clinical assessment), prognostic and therapeutic value (for the purpose of the choice of therapy based on the identification of the infectious agent and its sensitivity to antimicrobial medicines).

The incidence of sepsis is high throughout the world, with 1,200,000 cases reported each year in Europe, of which 157,000 are fatal [2]. It is possible, however, that these data are underestimated because of the use of blood cultures that are not always optimal for cultural and organizational reasons. The morbidity and mortality that can be attributed to sepsis in the general population are in any event high, so high that sepsis is among the top 7 causes of death in Europe and North America [2]. From an aetiological point of view, in recent years, there has been a reduction in cases of sepsis due to Gram-positive and methicillin-resistant Staphylococcus aureus and a parallel increase in Gram-negative infections (*Klebsiella, Pseudomonas*, *Escherichia coli*, *Acinetobacter*) and from *Candida* (in particular from *C. parapsilosis*, *C. glabrata* and *C. krusei*). Infections of the respiratory tract are the most common origin of sepsis in the western world (35% of the total), including a large number of infections of the lower airways and infection-related ventilator-associated complications. Urinary origin appears to be reducing, although this accounts for around a quarter of cases. A lower number of sepsis episodes originate in the gastrointestinal tract or the skin and soft tissues (11% each) [3,4].

In patients with sepsis, quick and accurate diagnosis of bacteraemia and fungaemia has a critical impact on the prognosis, because swift initiation of a targeted antibiotic therapy (within 24-48 hours) significantly increases the chance of survival (the lethality associated with infection is reduced by 20-30%), and prevents the indiscriminate use of anti-infective medicines, thereby reducing the economic costs and the risk of the development of resistance. Blood culture, therefore, is the best instrument for the diagnosis of sepsis in all its manifestations, and should be performed, in the event of clinical suspicion, as early as possible while implementing strategies of a technical, methodological and organizational nature to reduce the time for execution and the transmission of the results to the clinician. However, investigations carried out in the Italian context have brought to light a significant variability of conduct in the sampling methods and microbiological diagnostic procedures [5], highlighting the need for authoritative and standardized indications for the execution of blood cultures in our country, as well as adequate information and training for all the health personnel involved. Most of cases of sepsis occur in the community, and therefore involve general practitioners and accident and emergency departments. Therefore, it is essential that accident and emergency facilities are involved in the work of increasing awareness about the critical importance of the optimal execution of blood cultures in patients with sepsis.

Moreover, blood culture must only be performed in response to a clinical need in patients with suspected bacteraemia; the execution of routine blood cultures is generally not recommended. There are numerous signs and symptoms that can suggest bacteraemia, which should always be part of an overall clinical judgment, but the main indicators to take into consideration in the assessment of a patient with suspected bacteraemia or sepsis are:

- body temperature outside the normal range;
- focal signs of infection;
- anomalies in the heart rate (increased), blood pressure (reduced or increased) or respiratory rate (increased);
- shivering;
- increased or very low white blood cell count;
- appearance of confusion.

It must always be taken into consideration that the signs of sepsis may be minimal or absent in very small children and elderly patients [6]. In patients with possible sepsis, blood cultures should be carried out immediately after the appearance of clinical suspicion and before the administration of an antibiotic.

For clinical purposes and in relation to health costs, it is equally important that the correct procedure is executed for the entire blood culture process, to reduce contam-

ination of the sample and the incidence of "false positives". The frequency of contamination of blood cultures (defined as the isolation of a microorganism introduced in the culture during the sampling and/or the processing of the sample that was not present in the patient's blood at the time of the sampling or, in any event, not implied in the active infection [7]) should not be higher than 3%, in accordance with international recommendations [8]. The correct procedures for antisepsis of the skin and inoculation of the sample must be used to reduce the risk of introducing the most common contaminants in the culture (*Bacillus spp.*, *Corynebacterium spp.*, *Propionibacterium spp.*, coagulase-negative staphylococci, *Aerococcus spp.*, *Micrococcus spp.*) [7].

Many procedural aspects are critical for correct execution of blood culture, including the time of sampling, the quantity of blood samples, the transport of the vials to the microbiology laboratory, all procedures that, with regard to the above, are essential and should be standardized and properly performed. Starting with what is available in international guidelines, and using the knowledge and clinical experience of a board of experts in the field, this document is offered as a practical guide for the clinician on when and how to perform sampling for blood culture in a patient with suspected sepsis. In the first part of the document, the evidence present in literature and the indications of the main international guidelines are briefly reported; the second part gives practical recommendations provided by the board's experts for the correct execution of the various phases of the blood culture test. The value of this document lies in the fact that it includes the entire operational process, from blood sampling to the delivery of the sample for microbiological assessment, thereby providing the clinician with a complete, accurate guide for reference currently not yet available in the Italian and European literature.

2. EXECUTION OF BLOOD CULTURES: GENERAL CONCEPTS

Although the blood culture test is an apparently simple diagnostic procedure, it involves several professionals (the patient's doctor, a specialist in infectious diseases, microbiologist, intensive care specialist, pharmacist, clinical risk officer, etc.) as well as a series of methods and materials, the quality of which should be optimized and standardized. Most of the existing guidelines are dedicated to the criteria of clinical decision making and microbiological diagnosis; only minor indications are available as a reference for the entire operational process, from sampling for blood culture to the delivery for assessment.

The elements that influence this process, particularly those that with regard to the execution of sampling, inoculation of the vials and their transport for analysis to the microbiology laboratory, can be identified as follows:

clinical aspects:

- timing of the sample
- interval between samples
- relationship with antibiotic treatment

and **technical aspects**:

- products used for antisepsis of the skin and for the sampling
- technical method of sampling
- volume of blood to be sampled
- number of blood cultures performed
- methods of preservation and transport of the sample
- safety of the operators.

On whom and when to perform a blood culture

For **identification of a patient** with a suspected infection of the blood stream caused by bacteria or fungi, for which the execution of a blood culture is indicated, in addition to fever and shivering, other parameters must be taken into consideration, including blood pressure and heart rate, white blood cell count and the biological markers of inflammation (such as PCR, procalcitonin [PCT] and determination of lactates). Among these, determination of the PCT level appears to be particularly useful, especially in patients with Gram-negative infections; absolute and kinetic values of the change in PCT levels can help the clinician in the diagnosis and management of the antibiotic treatment [9,10]. Conversely, fever is itself absolutely not a discriminating factor, because some patients can be normothermic or hypothermic in the bacteraemic phase [11].

On the **choice of the most suitable time** to perform sampling, for the purpose of optimizing the probability of isolating pathogens from the blood, the experimental recommendations in the literature are limited. However, it has been demonstrated that the practice of performing sampling for blood culture on the appearance of shivering or a rapid increase in temperature does not increase the rate of positivity of the test [12], based on the observation that, after the entry of bacteria in the bloodstream, there is a delay of around an hour before the appearance of shivering or fever [13]. Even the common practice of obtaining the samples for blood culture at intervals of 30-60 minutes is not supported by the evidence, because differences have not been observed in terms of the capacity for microbiological isolation if all the samples are taken at the same time or at set intervals within the span of 24 hours [14]. For these reasons, most of the existing guidelines recommend execution of all blood cultures simultaneously or within a short period of time [7,11,15], especially when there is a clinical need to begin empiric antibiotic treatment [8]. The rationale for the use of a "single-sampling strategy" (SSS; that is, the entire volume of blood from a single sample is subdivided into 4-6 bottles) is based on the possibility of reducing the rate of contamination by limiting the number of samples, limiting the workload and occupational risk to the operators, reducing the costs and the discomfort for patients; for these reasons, this strategy was proposed as the method of choice by some authors [16]. The exceptions are patients with suspected subacute bacterial endocarditis or other endovascular infections (e.g. linked to a catheter), in whom the execution of blood cultures at regular intervals for a period of 24 hours (repeated when necessary in the case of negativity) may be useful to document continuous bacteraemia [7,11].

All the recommendations reiterate that blood culture should be performed before the start of empiric **antibiotic treatment**: unsuccessful microbiological isolation in cultures can occur within minutes or hours after the first dose of an antimicrobial. The isolation of a pathogen makes it possible to consider a subsequent de-escalation of antimicrobial therapy, with a reduction in the antibiotic selection pressure, less side effects and lower costs [17]. Various retrospective studies have suggested that obtaining blood cultures before the start of antibiotic treatment is associated with a better outcome for patients [18]. If, in balancing the risk and benefit, it is necessary to rapidly start an antibiotic treatment before it is possible to perform blood cultures in a critically ill patient, there are indications for the execution of sampling immediately before a new administration, when the concentrations of medicine in the blood is at a minimum [11].

Number of samples and volume of blood to be taken

For the purpose of isolating pathogenic germs, it has been demonstrated that the **volume of blood** sampled is more important than the timing and appears to be the most determinant variable in numerous studies conducted on adult patients with bacteraemia and fungaemia. Li et al. demonstrated that an increase in the volume of blood from 20 ml to 40 ml for culture increases the diagnostic yield by 20%, and an increase to between 40 and 60 ml leads to a further increase of 10%, irrespective of the method of collecting the sample (simultaneously or a series of samples within 24 hours) [14]. In general, in adult patients, the probability of isolating the pathogen in the case of sepsis increases in direct proportion to the volume of blood sampled from 20 to 30 ml, beyond which the increase is still present but no longer directly proportional to the volume cultivated [14]. The direct proportion between the volume of blood sampled and the probability of identifying pathogens also appears to be valid for paediatric patients, although the data available in this population are limited. As a consequence of these observations, the existing guidelines recommend **sampling of 20-30 ml of blood for each set (complete blood culture sampling: 4-6 vials with a single sample of 40-60 ml of blood)** in adult patients [7,8,11]; for young children, tables are available that relate the blood volume to be sampled to weight and age (Table 1) [8], always taking into consideration that this should not exceed 1-4.5% of the total blood volume of the patient [7,11].

The blood collected at every sampling is generally subdivided in order to inoculate 2 vials: one for the search for aerobic germs and one for anaerobic germs. This practice has been questioned in recent decades on the basis of studies that reported a reduction in the incidence of bacteraemia from obligate anaerobic bacteria, therefore suggesting the use of specific vials only in selected cases and routinely using only vials for aerobic germs [19,20].

Table 1. Blood volumes recommended for blood culture in paediatric patients [8]

Weight of patient (kg)	Total blood volume of the patient (ml)	Recommended blood volume for culture (ml)		Total volume for culture (ml)	% of the total blood volume
		First set of cultures	Second set of cultures		
≤1	50-99	2	–	2	4
1.1-2	100-200	2	2	4	4
2.1-12.7	>200	4	2	6	3
12.8-36.3	>800	10	10	20	2,5
>36.3	>2200	20-30	20-30	40-60	1.8-2.7

When 10 ml of blood or less is collected, a single bottle should be inoculated for aerobic germs.

However, these data have never been validated, in particular with regard to the identification of patients for anaerobic culture; on the other hand, better identification of *Staphylococcus* and members of the Enterobacteriaceae family has been reported, in addition to anaerobic germs, with the use of a combination of vials for aerobic/anaerobic germs [21]. Currently, therefore, the recommendations in the guidelines suggest the use of the 2 traditional types of vials for adult patients [7]. For paediatric patients (for whom appropriate vials are also available requiring reduced quantities of blood), a single vial for aerobic germs can be inoculated when the volume of the blood sampled is less than 10 ml [8]. Because infections due to anaerobic bacteria are rare in children, some authors recommend the use of vials for aerobic germs only in the paediatric population, reserving vials for anaerobic germs for high risk patients (chronic oral or sinus infections, cellulitis, patients with abdominal signs and symptoms, bite wounds, septic phlebitis and neutropenic patients being treated with steroids) [7]. Moreover, because pathogenic fungi are cultivated usually only in vials for aerobic germs, for patients with suspected fungaemia, unless specific vials for the growth of fungi are used, some guidelines recommend taking at least one set of blood culture samples using 2 vials for aerobic germs rather than the classic combination of aerobic and anaerobic germs [8].

A second determinant to ensure greater sensitivity of the test is the number of blood culture sets performed during a septic episode. Generally, in adults with suspected bacteraemia, a **minimum of 2 and a maximum of 3** [7,11] ***or 4*** [8] **sets of blood cultures** (4-6/8 vials) at more or less close intervals (or even simultaneously, as discussed above) is recommended. The execution of a single sample in adult patients is strongly discouraged due to insufficiency of the volume of blood analysed, with the consequent increase in false-negative results and the difficulty in identifying false positives [11]. These indications are based on the results of various studies that have investigated the optimal number of blood cultures necessary to identify bacteraemia or fungaemia, reporting a probability of isolating the pathogen in cases of sepsis equal to 80-91% with a single sample and 99% and more with 2 or 3 samples [22,23]. More recently, using automated culture systems (CMBCS) rather than manual ones, percentages of 65%, 80% and 96% have been observed with 1, 2 and 3 sets of blood cultures, respectively [24]. It is possible that the lower sensitivity observed in the latter study may be connected to the different definitions of a true positive and contaminated culture, in addition to the various systems of culture used [7].

The guidelines do not generally recommend the routine execution of single blood cultures as surveillance culture (a practice often followed in patients admitted to intensive care or haematology departments) because they are considered of little clinical value and associated with an increase in costs [11]. Nevertheless, because the incremental costs are not significant, as part of the management of a critically ill patient, it may be appropriate to favour the clinical value of a positive blood culture that might potentially not be detected.

It is commonly recommended that sampling for blood culture is performed with venous blood because the use of arterial blood adds nothing to the sensitivity of the test [7,11]. In patients with venous catheters, the guidelines unanimously indicate that these should not be used to take blood samples, except in patients where an infection associated with the catheter itself (catheter-related bloodstream infection, CR-BSI) is suspected, in which case a peripheral sample and one from the catheter must be taken in parallel [7,11]. However, in real life, especially in certain categories of patients, such as those admitted to intensive care units or haematology, it can be difficult or even impossible to access a peripheral vein or obtain a sufficient quality of blood from venous sampling, and it is therefore necessary in various cases to resort to sampling from a central venous catheter (CVC), irrespective of suspected CR-BSI. Peripheral venous blood sampling, compatible with the clinical conditions, nevertheless remains the gold standard for the correct execution of blood samples. In the absence of a suitable peripheral venous access, it is also possible to use arterial blood, which does not present any demonstrated disadvantages compared with venous blood in terms of contamination and sensitivity.

How to perform a blood culture: operating methods

Antisepsis of the skin is a critical measure initially to reduce the risk of sample contamination. All the existing guidelines pay significant attention to this aspect because contamination and false positives are a prob-

lem with significant clinical and economic impact. Contamination can occur from several sources, including the patient's skin, the aids and devices used to take the sample and transfer it to the vials for culture, the operator's hands and the surrounding environment [6]. The most frequent cause of sample contamination is the lack of or improper antisepsis of the skin, even though a small percentage of bacteria that live in the deep layers of the skin can survive antisepsis, especially when located between the skin folds where the antiseptic does not penetrate.

Over the last 50 years, numerous antiseptic agents have been used clinically, including alcohol (ethyl or 70% isopropyl), iodine tincture, povidone-iodine, iodophors and chlorhexidine gluconate. The conclusions of a systematic review published in 2007 to compare the effectiveness of these agents were as follows [25]:

- iodine tincture (hydroalcoholic) is more effective than aqueous povidone-iodine solution in reducing the risk of sample contamination;
- an alcohol solution of chlorhexidine gluconate is more effective than aqueous povidone-iodine solution;
- alcohol antiseptics are more effective than aqueous solutions;
- alcohol alone is not inferior to iodine-based solutions.

Furthermore, the authors have stated a possible benefit arising from the use of pre-packaged antiseptic kits [25]. A more recent meta-analysis of 6 clinical trials concluded that an alcohol solution of chlorhexidine is more effective than an aqueous povidone-iodine solution in the reduction of false positives in blood culture tests and that alcohol solutions are more effective than non-alcohol solutions, even though the comparison between chlorhexidine-based and iodine-based products is not yet conclusive [26]. A recent study that compared the effectiveness of 3 antiseptics (10% aqueous povidone-iodine solution, 2% iodine tincture and 2% chlorhexidine in 70% isopropyl alcohol) confirmed the importance of the execution of the procedure by expert personnel; this can reduce the total sample contamination rate, irrespective of the antiseptic used [27].

In clinical practice, when using povidone-iodine, it is important that operators respect the 2 minutes necessary to reach the maximum antimicrobial effect. Conversely, chlorhexidine is a rapid action agent (30 seconds is sufficient) and persists on the skin. Moreover, it is rarely associated with allergic reactions (any minor reactions of hypersensitivity must, in any case, be reported and the subsequent use of chlorhexidine must be avoided) [28] and the skin does not need to be cleaned after taking the blood sample. Therefore, although the data in the literature are sometimes not in agreement on the execution of sampling for blood culture in adults and children (>2 months old), most of the guidelines suggest the use of 2% chlorhexidine in alcohol solution [6,7,28]. Although the guidelines do not recommend sampling of blood for blood culture from venous catheters, when this must be performed (e.g. in patients with suspected bacteraemia associated with the catheter), the antisepsis methods are similar: application of 2% chlorhexidine gluconate in 70% isopropyl alcohol for the disinfection of taps or access ports. In patients with sensitivity to chlorhexidine, povidone-iodine in alcohol solution can be used [28].

The **difference between "medicines" and "biocides"** should also be taken into consideration. Although an antiseptic intended for use on intact skin can be registered as a medicine or as a biocide, the production, distribution and registration process of the 2 classes of agents is significantly different and can give rise to concerns about the use of biocides for medical purposes (the matter is explored in depth in the Appendix "Medicines vs biocides").

In addition to the choice of antiseptic agent, the technique used to apply the antiseptic is equally important for correct antisepsis of the skin [29]. There are precise indications in the literature on the execution of antisepsis: apply 2% chlorhexidine in 70% isopropyl alcohol while scrubbing the skin over an area 7-8 cm in diameter for 30 seconds and then wait for around 30 seconds for the skin to dry. The traditional method of applying antiseptic in concentric circles starting from the injection site outwards came into question, and forward and reverse rubbing is now considered the best approach to remove the layers of skin and so more effectively reduce the bacterial load of the epidermis [30]. Moreover, there is evidence in favour of the use of **sterile devices in disposable packaging**, preferably registered as a medicine, which avoid contamination of the antiseptic itself and provide numerous other advantages in terms of exact dosage, standardization of the procedure and mechanical action guaranteed by the application device [6,28,31].

To avoid possible contaminations, the guidelines emphasize the following:

- **disinfect the vial stopper**, which is not sterile, before inoculation (with a specific product with similar composition to the product used for antisepsis of the skin, leaving it to act for the same time, or with 70% isopropyl alcohol);
- **wear disposable gloves**, not necessarily sterile unless it is necessary to repeat palpation of the sample site after antisepsis of the skin to localize the vein [7,11].

The use of gloves is an essential part of clinical practice for health operators and has 2 main purposes:

1. to protect the hands of the operator from contamination by microorganisms, blood and bodily fluids;
2. to reduce the risk of transmission of microorganisms to the staff and the patients.

In the execution of sampling for blood cultures, wearing disposable gloves of a suitable size is a practice aimed at reducing the risk of contamination and guaranteeing the operator's safety. However, even the use of gloves must be done correctly and must not have a negative impact on hand hygiene; some studies have reported reduced hygiene among operators when gloves are used [32,33]. The international guidelines reiterate the need for hand hygiene before gloves are worn and immediately after their removal [6,28]. The indications for the use of gloves provided by the Epic3 guidelines are summarized in Table 2 [28].

Table 2. Correct use of gloves

GLOVES MUST BE:
▪ used as a disposable instrument
▪ worn immediately before contact with the patient or execution of the procedure
▪ removed as soon as the procedure is completed
▪ changed when passing from one patient to another
▪ thrown away in the correct waste container in accordance with local policy

Sampling must be done with a vacuum system using a device with a safety mechanism to protect the health operator from needle puncture.

Recently, the use of initial specimen diversion devices (ISDDs) has been reported, which capture the first 1.5–2 ml of sampled blood (which presumably contains skin cells and contaminating microorganisms) to reduce contamination in blood culture samples: in a study conducted on 971 individuals admitted to emergency departments, the use of ISDDs was shown to reduce the contamination rate from 1.78% with the standard procedure to 0.22% [34]. However, further studies will be necessary to validate this technique and confirm that there is no increase in the risk of haemolysis of the sample or greater contamination rates.

When the same sample is used for purposes other than for blood culture, the vials for blood culture must be inoculated first in order to avoid contaminations [11,35].

The correct sampling procedure for blood cultures also includes all the regulations necessary to preserve the **safety of the operator** (Decree 19/2015, incorporated in Directive 32/2010/EU; Heading X bis of Legislative Decree 81/2008 and subsequent amendments and supplements).

To help understand the nature of the problem, there are more than 60 pathogens that can be transmitted via the blood (including hepatitis B, hepatitis C and HIV) and the practices with the greatest risk of infection are those that involve the use of hollow needles. Accidental puncture with needles or injury with sharp objects is a frequent occurrence among health operators; in Europe, around 1,200,000 accidents a year are reported (Directive 2010/32/EC).

In the national context, a study by AIRESPSA (*Italian Association of Protective and Prevention Services Managers in the Healthcare Environment*) showed that accidents with biological risk from blood-transmitted diseases (punctures, cuts and mucocutaneous contamination) represent around 40% of all accidents in hospital environments [36]. According to the data from the SIROH project (*Italian Study on the Occupational Risk from HIV and other blood-borne pathogens*), two thirds (65%) of accidental punctures among health operators in Italy can be attributed to hollow needles filled with blood, and 42% of seroconversions to at least one of the HIV, HBC an HCV viruses occur following puncture with a needle while taking a blood sample [37]. Furthermore, in a survey in 2001, of a total of 439 accidental punctures by needles, 74% were caused by the incorrect conduct of

the operator and only 26% were not preventable [38]. With regard to adherence by Italian health personnel to EU Directive 2010/32, a recent study conducted in 100 SIROH hospitals specifically engaged in prevention, a good rate of adherence to the preventive measures in the Directive (in particular, needles should not be capped and operators should receive specific training) was reported, although with the need to further implement the use of safety instruments [39]. Further studies will be needed to assess the situation in other Italian hospitals, but it is clear that the issue of safety in the execution of blood culture sampling has significant epidemiological relevance and needs to be tackled with the correct procedures and training of health personnel.

Inoculation of the vials and transport of the samples

The **volume of blood introduced to each vial** for blood culture is once again a critical variable for the diagnostic efficiency of the test. The need to inoculate the right quantity of blood arises from the numerous substances present in human blood that can inhibit microbial growth (complement, lysozyme, phagocytes, antibodies, etc.): in order to reduce the concentrations of these factors and minimize their inhibitory activity, the blood must be diluted in the culture medium to a ratio of between 1:5 and 1:10 [7]. Generally, it is recommended that around 8 ml of blood should be added to each vial, but not exceeding 10 ml [11]. In cases of problems linked to venous sampling, the volume of blood inoculated must not be less than 5 ml. Excessive volumes (>10 ml) can lead to false-positive or false-negative results. Keeping the bottles in an upright position can be a useful method to ensure that the right quantity of blood is inoculated [6,11].
As discussed above, it is currently recommended to **inoculate at least 4 vials** (2 for anaerobic germs and 2 for aerobic germs), although, **in the absence of technical difficulties or other problems, the inoculation of 6 vials can be considered optimal** for diagnostic purposes. The use of selective media for the culture of fungi is still an open question. The guidelines of the Italian AMCLI (*Italian Association of Clinical Microbiologists*) do not acknowledge that there is sufficient scientific evidence for the use of selective media for fungi. These require longer processing times, in addition to the aerobic/anaerobic sets for routine searches, but in the case of investigations for immunoexpressed/nonhematological patients, these vials could be useful to detect the presence of fungi masked by a bacterial superinfection. According to the same guidelines, however, media containing resins could better promote the growth of staphylococci (including contaminants) and fungi [11]. Moreover, they may be useful in identifying microorganisms from the blood of patients being treated with antibiotics [7].

The transport of inoculated vials to the laboratory for incubation and the **time** are an integral part of the correct execution of the test and involve technical and organizational aspects. All microbes grow, multiply and die very rapidly. If each of these events occur during sampling, transport or preservation of the sample, the results of the analysis will be compromised and their interpretation could be wrong. Attention to the pre-analytic management of microbiological samples, especially regarding their arrival in the laboratory as quickly as possible after sampling, is therefore critical for the accuracy of the test [8].
According to the guidelines, the vials should be incubated immediately but, in any case, within 1 hour of sampling, because any delays could delay or prevent microbial growth. While awaiting transport to the laboratory, the blood culture vials should be preserved at optimal temperatures, not too high (no higher than 30°C) and never refrigerated at 4°C or frozen [7,11]. Some guidelines suggest the vials can be kept at ambient temperature for a maximum of 16-18 hours in particular cases, although it is emphasized that retention for long periods outside automatic incubation systems can cause a lack of seroconversion of the vial by the instrument. Microbial growth could have already reached the stationary phase outside the system and therefore may not be detectable, and microbial distress may have occurred, with consequent lack of growth of microorganisms [11]. Keeping to the optimal times requires sufficient departmental organization to enable the samples to be sent to microbiology as soon as they are taken (one daily "shipment", for example in the evening, should be avoided). These problems can be especially evident in accident and emergency departments, which, as mentioned previously, play a decisive role in the identification of the aetiological agent before the start of antibiotic treatment. In these departments,

and when it is not possible to guarantee delivery within the optimal times, if the hospital facilities do not have a round-the-clock microbiology service 7 days a week (rare in Italy), the use of delocalized incubators can be considered. Using this type of approach, a significant time advantage has been demonstrated in terms of obtaining a microbiological result and the start of an appropriate antibiotic treatment [40]. An alternative solution reported by some authors is placing of an incubator outside the laboratory to allow direct incubation of the vials outside the laboratory's opening times [41]. In both cases, adequate training and awareness of the departmental personnel who perform the blood cultures are necessary.

3. CORRECT PROCEDURES FOR EXECUTION AND TRANSPORT OF SAMPLES FOR BLOOD CULTURE

As highlighted in the discussion on the various procedural aspects of the execution of blood cultures, for the purpose of:

- obtaining a rapid and accurate diagnosis
- avoiding diagnostic errors linked to false positives and false negatives

it is essential that the entire process, from the preparation to the execution of sampling, the preservation and transport of the samples, is standardized in detail and correctly performed.

A **practical guide** for the correct execution of the various phases of the blood culture test is given below.

➤ PREPARATION FOR SAMPLING

RECOMMENDATIONS

- **Sampling for blood culture must be performed by health operators, possibly in pairs, who are expert and competent in the various phases of the procedure.**
  Ideally, following the example of Anglo-Saxon countries, the training of a dedicated nursing team should be supported for the execution of blood cultures (following the example of the "PICC teams"). When this is not possible, it is necessary to provide adequate training for the entire nursing personnel.

- **The procedure must be carefully standardized and registered.**
  It is to be hoped that every health facility will define a protocol to follow, with a complete checklist of all the steps to be completed. All blood cultures performed must be documented in the patient's medical record, registering the date, time, sampling site and indications [6].

- **Sampling should be organized carefully by preparation and control of all the material necessary on a trolley, on a sterile cloth (Table 3).**

- **Immediately before sampling, the operator must carry out careful hand hygiene, performing antisepsis with a hydroalcoholic preparation and wearing disposable gloves.**
  It is important to remember that the use of gloves is not an alternative procedure to proper hand hygiene. Hand hygiene must be performed (preferably by antiseptic rubbing with hydroalcoholic gel) before gloves are worn and after their removal.
  The use of **sterile** gloves is not strictly necessary. **Non-sterile** disposable gloves of the right size can be used as long as great attention is paid to avoiding touching the area identified for sampling after the execution of antisepsis (e.g. to palpate the vein to be punctured).

- **The blood sample for blood cultures must, as a general rule, be taken from a peripheral vein, avoiding the use of samples from pre-existing peripheral cannulas or central catheters (e.g. port-a-cath or CVC), which carry a greater risk of contamination.**
  However, there are clinical situations, especially in intensive care or haematology departments, when it may be difficult or impossible to access a peripheral vein or obtain a sufficient volume of blood. In these cases, if a pre-existing peripheral or central catheter is used, due caution must be exercised in interpreting the results. A suitable venous site must be identified before proceeding to antisepsis of the skin. In the absence of suitable peripheral venous access, it is also possible to use **arterial blood**, which does not present any demonstrated disadvantages compared with venous blood in terms of contamination and sensitivity.

A sample from a catheter, always in association with a peripheral venous sample, possibly contralateral, must be taken in cases of **suspected bacteraemia or fungaemia associated with the catheter** (CR-BSI). In these cases, 2 sets of blood cultures must be obtained at the same time, one from a peripheral sample and one from the catheter, suitably marked with the sample source. Various microbiological methods have been developed to interpret the results obtained and confirm or otherwise the presence of bacteraemia or fungaemia associated with the catheter (Table 4) [7,11].

Table 3. Preparation of material for blood culture sampling

MATERIAL FOR BLOOD CULTURE FROM A PERIPHERAL VEIN
▪ Disposable device for antisepsis of the skin based on 2% chlorhexidine gluconate in 70% isopropyl alcohol, preferably registered as a medicine
▪ Safety butterfly and jacket for sampling
▪ Culture media for aerobic, anaerobic germs and any fungi
▪ Tourniquet
▪ Non-sterile gauze
▪ Individual protective devices (mask, sterile and non-sterile gloves, face shield in the event of blood splatters, particularly when collecting a sample of arterial blood)
▪ Containers for hospital waste with infective risk (including one for the disposal of sharp objects)

Table 4. Diagnosis of infection correlated to a catheter based on the use of comparative blood cultures [11]

RESULTS OF THE BLOOD CULTURES		INTERPRETATION
Isolation of the same strain from a CVC and peripheral vein	Significant load or growth times	Strongly suggestive of CVC-correlated infection in the absence of other infection sources
	Insignificant load or growth times	Suggestive of possible CVC-correlated infection in the absence of other infection sources
Positive only from CVC		Inconclusive for CVC-correlated infection. Possible colonization of the catheter or contamination during collection
Positive only from peripheral vein		Inconclusive for CVC-correlated infection. However, suggestive in case of isolation of *S. aureus or Candida spp.*, in the absence of other sources of infection
Negative from CVC and from peripheral vein		Catheter-correlated infection: improbable

➤ ANTISEPSIS OF THE SKIN

Antisepsis of the skin must be performed rigorously at the time of the sampling in order to avoid contamination of the sample and the consequent alteration of the result of the test. A contamination rate of blood cultures of 3% is generally considered the maximum acceptable value [8]. The microbiology laboratory of every health facility should analyse contamination data of blood cultures at set time intervals (e.g. every 6 months) and discuss them with the clinical team, if necessary.

RECOMMENDATIONS

- **As indicated by the guidelines and validated by the literature, antisepsis of the skin must be performed using 2% chlorhexidine in 70% isopropyl alcohol.**
  The pre-selected site should be disinfected by scrubbing the skin over an area 6-7 cm in diameter for 30 seconds, then waiting for around 30 seconds for the antiseptic to dry.
  In the (rare) patients allergic to chlorhexidine, 10% povidone-iodine can be used for 120 seconds. The use of chlorhexidine is not recommended in newborns less than 2 months old.
  When a venous, peripheral or central catheter is used for sampling, great attention must also be paid to disinfecting these instruments, using a specific product with a similar composition (2% chlorhexidine in 70% isopropyl alcohol) to carefully clean the valves. These should be treated for a minimum of 30 seconds and left to dry before using the system.
- **The use of sterile disposable devices, preferably registered as medicines, is recommended to avoid contamination of the antiseptic.**
  The use of these systems has various advantages in terms of standardization, exact dosage, sterility and mechanical action, resulting in greater effectiveness of antisepsis. A significant reduction in contaminations of blood cultures has been demonstrated with the use of these devices [31].
- **Cleaning the skin treated with chlorhexidine after execution of the sampling is not necessary.**
- **The vial stopper is not sterile and this must also be disinfected before inoculation.**
  For the disinfection of the vial stopper, a specific product can be used with a similar composition as the antiseptic used for the skin (2% chlorhexidine in 70% isopropyl alcohol) or 70% isopropyl alcohol alone.

Table 5. Correct practices for the prevention of the infections (modified by [35])

YES	NO
▪ Perform careful hand hygiene (soap and water or rubbing with alcohol), including the wrists and the spaces between the fingers, for at least 30 seconds	▪ Do not forget hand hygiene
▪ Use a pair of non-sterile disposable gloves for each procedure or patient	▪ Do not use the same pair of gloves for more than one patient ▪ Do not wash gloves in order to reuse them
▪ Disinfect the skin at the sampling site before performing the procedure	▪ Do not touch the sampling site after disinfecting it
▪ Immediately dispose of the device used for the sampling in a container for sharp objects	▪ Do not leave an unprotected needle outside the container for sharp waste
▪ Close the container for sharp waste with a suitable cover	▪ Do not overfill the container for sharp objects
▪ Place test tubes and vials for laboratory tests in a resistant container before transferring the blood sample	▪ Do not inject blood into a test tube

➤ VOLUME OF BLOOD TO BE SAMPLED

The volume of blood that is sampled for every set of blood cultures (defined as all the vials that are inoculated with a single peripheral sample or from a catheter) is the most important variable for the diagnostic outcome of the test in patients with suspected sepsis.

RECOMMENDATIONS

- **The volume of blood to be sampled for each set must be at least 20-30 ml, to be subdivided in different vials (at least 1 for aerobic germs and 1 for anaerobic germs). The complete blood culture sample is made up of at least 2 sets.**

  Notwithstanding its importance, this parameter is little respected in practice, especially when there are problems in obtaining a sample of peripheral blood, with the consequent arrival in the laboratory of vials not correctly filled and significant problems with the microbiological interpretation of the results.

- **In the subdivision of the sample into different vials, those for aerobic germs should be filled first and then those for anaerobic germs; also, in the case of several samples from the same venepuncture, the blood culture samples must be performed first.**

- **Every vial must be inoculated with an optimal volume of blood (8-10 ml). When there are problems with venous sampling, no less than 5 ml of blood should be inoculated.**

  Inadequate filling of the vials poses significant diagnostic problems. An insufficient volume can cause false-negative results or delay in the growth of microorganisms. Overfilling the vial, moreover, can lead to false-negative results (due to the formation of small clots that trap the microorganisms) or false-positive results (due to high levels of leucocytes).

  To obtain an optimal volume of inoculation, it is important to indicate the volume required on the vial and hold the vial in an upright position during the inoculation (e.g. avoiding placing the vial horizontally on the patient's bed).

  The volume should be checked by the operator who performs the sampling in the department but an additional control, at least visual, must be carried out by the microbiology unit before the incubation of the sample.

- **At least 4 vials should be inoculated (2 for anaerobic germs and 2 for aerobic germs) for each patient with suspected sepsis. Where possible, the inoculation of 6 vials can be considered optimal.**

  When there are no difficulties with venous sampling and no other problems, the inoculation of 6 vials can be considered optimal when a sufficient volume of blood is obtained.

  With regard to the **sampling time**, 2 different approaches have been described with comparable effectiveness: the first is the standard approach (multi-sampling strategy) based on the execution of at least 2 sets of samples within a short time (within 10-12 minutes); the second (single-sampling strategy), introduced more recently, is based on the execution of a single sample for blood culture with inoculation of all the vials at the same time (4-6 in total) [14-16].

The second approach has the advantage of being able to collect a sufficient volume of blood with a single sample and with the simultaneous filling of at least 4 vials. It is associated with a reduction in the risk of skin contamination linked to several samples, a reduction in the workflow and less risk of exposure to pathogens; the possibility also exists of initiating an empiric antibiotic treatment earlier without having to wait for other blood culture samples. **In general, the single-sampling strategy seems to be preferable in some patients, such as those in intensive care.**

Sampling should be performed on patients with a fever or, in any event, with clinical suspicion of sepsis, at any time, without the need to wait for the appearance of shivering and/or the peak of the fever and possibly before the start of antibiotic treatment.

In patients with subacute **endocarditis** or with other particular clinical conditions (e.g. bone infection), the execution of 3 sets of blood cultures at intervals of around 15-30 minutes is recommended and, in the event of negative results, another 3 sets after 24 hours.

➤ SAMPLING TECHNIQUE AND SAFETY

Materials and techniques must be used for the execution of sampling for blood culture that guarantee the operator's safety, taking into consideration that accidental puncture with needles is a significant proportion of the accidents recorded in hospitals [36]. In particular, the field of blood culture sampling carries a high risk because the patient is probably a carrier of pathogens that can be transmitted to the operator. It is therefore imperative to use the latest generation of safety devices.

RECOMMENDATIONS

- **In accordance with the international recommendations and the European Directive, the use of syringes must be avoided and devices must be used that guarantee the safety of the patient and the health operator: a set for sampling with a butterfly needle equipped with a safety mechanism and adapter for multiple sampling for the collection of blood directly into the vials.**
  The practice of capping sharp objects has been prohibited since 1990 and banned by Directive 2010/32/EU and then by Decree 19/2015. At the end of the blood sampling, activate the safety mechanisms and dispose of the sampling set in the appropriate container for sharp waste.

Table 6 gives the complete list of the important guidance criteria for the correct definition and assessment of a device for the prevention of accidental punctures (NDPS), established by numerous international agencies, the government of the Autonomous Community of Madrid and by the National Institute for Insurance against Accidents at Work.

Table 6. Specifications of the safety devices suggested by the international agencies

GUIDANCE CRITERIA FOR THE CORRECT DEFINITION AND ASSESSMENT OF A DEVICE FOR THE PREVENTION OF ACCIDENTAL PUNCTURES (NDPS)
▪ The protection mechanism must preferably be activated automatically (active or passive trigger) and, in any event, with only one hand
▪ The operator's hands must always be behind the sharp part of the device
▪ The protection mechanism must be activated as soon as possible
▪ The device must be reliable, easy to use and intuitive
▪ The protection mechanism must create an effective, permanent and irreversible barrier between the sharp part of the device and the operator
▪ The protection mechanism must not be able to be deactivated and its protective function must be guaranteed even during and after disposal
▪ The device must be furnished with a signal (audible and/or visible) that confirm the activation of the protection mechanism
▪ The protection mechanism must be an integral part of the device and not an accessory
▪ The use of the device must not generate additional risks to safety (e.g. the risk of mucocutaneous exposure)
▪ The device must in no way compromise the quality of the intervention and the safety of the patient

➤ MEDIUM TO BE USED

In addition to the indication to always use 2 types of media, 1 for aerobic germs and 1 for anaerobic germs, it remains optional to use a third vial for fungi and vials with special media (enriched with substances that improve microbial recovery by absorbing antimicrobial substances and that lyse white blood cells to release the microorganisms in the mixture).

RECOMMENDATIONS

- **In most patients, the use of a third vial specifically for fungi is not necessary because many fungi also grow in the aerobic and anaerobic vials, with lower processing times (5 days rather than 14 days).**
  In patients at risk of *Candida*, in particular, the use of a third vial specifically for yeast may be superfluous: many species of *Candida* grow well in media for aerobic germs, and *C. glabrata* grows in media for anaerobic germs.
  However, a specific vial for fungi can be useful in certain cases, such as immunocompromised patients (haematological patients, with HIV, etc.) at risk of infection from filamentous fungi (e.g. *Aspergillus*, *Fusarium*).
- **Although blood cultures should not be performed after the start of antibiotic treatment, it has been shown that it is still a widespread practice, just as the volume of blood to be sampled is often not sufficient for the good practices described above (incomplete volumes). Reiterating that it is appropriate to follow good practices and therefore avoid these situations, the use of vials with resins is a valid practice to ensure correct analysis of blood culture tests also under these conditions.**
  Media with added resins can better support the growth of certain microorganisms (e.g. staphylococci), giving the possibility of a diagnostic response even in situations where the volume of inoculated blood is not adequate according to the good practices described above.
  A valid alternative to the use of media containing resins can be rigorous respect for the blood, i.e. a culture medium dilution ratio (generally 1:5) sufficient to dilute antimicrobial agents.

➤ SAMPLE TRANSPORT TIMES

The time to transport the inoculated vials to the laboratory for incubation is a critical factor for the correct execution of the test, because any delays beyond the maximum limit, generally 1 hour, can delay or prevent microbial growth.

RECOMMENDATIONS

- **The inoculated vials for blood culture must be sent immediately to the microbiology laboratory.**
- **A maximum interval of 1 hour between sampling and incubation of the sample can be considered optimal. The maximum acceptable limit is 4 hours: beyond this time span, the significance of the test is highly compromised.**
  In order to be able to follow these recommendations, health facilities must have adequate organization in place so that the vials can reach the microbiology laboratory within a short interval of time. For example, the practice of accumulating blood culture samples performed in the course of a day and sending them all together to the laboratory at the end of the day is not acceptable.
- **The inoculated vials for blood culture must be preserved at optimal temperatures (no higher than 30 °C) and not refrigerated or frozen while waiting to be sent to the laboratory.**
- **In health organizations where it is not possible to guarantee that the vials will be sent to microbiology within 1 hour, the use of delocalized incubators is advisable.**
  Delocalized incubators can be a useful resource in accident and emergency departments and intensive care units where there are often problems at this phase of the procedure, and in hospitals that do not have a microbiology service active 24 hours a day, 7 days a week. Although 24-hour availability is very rare in Italy, microbiology laboratories opening for only 6 hours a day is not compatible with the service required; all microbiology services should guarantee opening hours of at least 12 hours a day, with some main centres open for 24 hours a day.
  Many microbiology laboratories in Italy are incorporated within the biochemical/clinical pathology laboratory, thereby ensuring longer service hours, but also posing the problem of the microbiological expertise of the personnel who handle the blood cultures. This practice can be a critical problem, especially in the absence of specific training of the laboratory personnel, and is in contrast with the exclusive expertise of the clinical microbiologist necessary for all the phases of the procedure (from the reading of the Gram microscopy test to the reporting).

Good practice for the execution of blood cultures (bundle)

- Hand hygiene
- Collect all the material necessary and place it on a clean tray (checking the expiry dates of all the articles to be used)
- Mark the optimal filling volume on the vials
- Wear gloves
- Apply the tourniquet
- Perform antisepsis of the preselected site for the sampling with 2% chlorhexidine in 70% isopropyl alcohol (disposable sterile applicator) for 30"
- Leave to dry for 30"
- Do not palpate the vein again (if the manoeuvre is necessary, wear sterile gloves)
- Remove the stopper from the vial for blood culture and disinfect (2% chlorhexidine in 70% isopropyl alcohol)
- Leave to dry for 30"
- Perform the sampling using a safety butterfly needle with adapter for the collection of blood directly in the vials
- The complete blood culture sampling is made up of at least 4-6 vials (2-3 sets)
- Keep the vial upright under the patient's arm
- Fill every vial with around 10 ml of blood (adult patients), first filling the aerobic vial and then the anaerobic one
- Remove the tourniquet as soon as the blood begins to flow or within 2 minutes 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 haemostasis with a dry swab
- Remove the gloves and perform hand hygiene
- Mark on the vials all the information necessary (number of the sample, site of the sampling, time and date)
- Indicate the execution of the blood culture in the patient's medical record
- Immediately send the vials to the laboratory
- If available, immediately place the vials in the delocalised incubators

Amended by [6].

4. CONCLUSIONS

The critical value of blood cultures in the clinical management of the patient with suspected sepsis is undeniable, but this value depends on the rapidity and accuracy of the microbiological report provided. In order to ensure this level of quality, microbiological samples must be suitably selected, sampled and transported, a process that involves various health personnel able to operate in accordance with good clinical practice within a multidisciplinary team. As highlighted in this document, the first to consider the entire process from taking the sample to its incubation, the correct execution of a blood culture involves various phases, each of which must be performed correctly to optimize the analysis and interpretation.

Alongside the professional clinical personnel, doctors and nurses, engaged in the diagnostic process, the role of the pharmacist must not be forgotten. The quality and availability of the medicines and medical devices required for correct blood culture practice undeniably have an impact on the validity of the test and the hospital pharmacist is responsible for their procurement, proper preservation and, together with the other personnel involved, the choice of pharmaceutical items, it is advisable to increase awareness in this field. In general, the need to implement a multidisciplinary effort and strategy at several levels is undeniable in order to improve the quality and reliability of blood sampling intended for blood culture.

Despite the existence of national and international guidelines, there is still a problem adapting to the recommendations in many Italian health organizations. In many hospital departments, the execution of blood cultures is insufficient in numerical terms and often incorrect, with a particular problem within accident and emergency departments. Although most cases of severe sepsis come from the community, making the involvement of the accident and emergency department essential, shortcomings of a cultural (often, there is a delay in transferring the patient to the department, but empiric antibiotic treatment is initiated) and organizational nature (lack of microbiology laboratories open 24 hours a day or of delocalized incubators that enable sampling to be performed at any time) make the execution of blood culture tests suboptimal in this setting.

It is therefore clear that organizational and cultural interventions are necessary to bring about improvements in clinical practice. In particular, with regard to cultural and educational aspects, certain potentially effective actions are advisable:

- dissemination and implementation of the guidelines. The production and dissemination of documents like this one is especially useful, bringing together all the operative manoeuvres for a blood culture test for the first time;
- interventions involving communication and training of medical and paramedical personnel (drawing up and disseminating authoritative documents, courses, but also practical training at the patient's bedside, bringing together all the professionals involved: doctor, nurses, microbiologist and others);
- the formation, within the hospital, of a sepsis team made up of nurses, clinical microbiologist, infectious diseases expert, who have expertise in sampling and, as a second step, the establishment and review of the treatments, involving the other professionals (intensive care expert, clinician, hospital pharmacist, etc.).

For these actions to be put into practice, it is vital to increase awareness among health managements 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. The medical-legal problems and health costs associated with the accuracy and reliability of blood culture tests should also be underlined. The incorrect execution of a blood culture 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 an increase in health costs but also in a pointless and damaging selective pressure, on the basis of the emergence of antimicrobial resistance.

Finally, extension of awareness of the problem of sepsis is to be hoped for beyond the strictly health care field, among institutions, the media and the general population. Indeed, it is known that 80% of septic events arise in communities [4]. Increasing awareness and educating people to recognize the initial symptoms means a reduction in fatal outcomes due to late interventions.

Sepsis is a global problem that needs to be adequately considered and tackled with a multidisciplinary approach. To guarantee the clinical effectiveness of blood culture, the main diagnostic test for bloodstream infections, and the best possible outcome for the patient, it is vital that the involvement of the various professionals is based on constant communication and a strict adherence to shared procedures. These aspects of communication and adherence to univocal protocols are currently the main problems and must be tackled 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 caused by sepsis in the global population.

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APPENDIX – MEDICINES VERSUS BIOCIDES

Overview of the issue

The market for antiseptics and disinfectants is broad and differentiated, especially in relation to regulatory authorization for production and release on the market.

DEFINITIONS

- ***Antiseptic:*** **organic or inorganic substance used on living tissues to prevent or stop the action and growth of pathogenic microorganisms.**
- ***Disinfectant:*** **chemical agent with antimicrobial activity intended for use on inanimate objects or surfaces (instruments or environmental).**

The European Chemicals Agency (ECHA) has provided a guide to the application of the European Regulation on matters of biocides (BPR – EU Regulations 528/2012). This indication specifies clearly that products for antisepsis of injured skin (e.g. antisepsis of a surgical wound) or for the antisepsis of intact skin before an invasive medical treatment (e.g. pre-operative antisepsis of the skin before a surgical intervention or before vascular access) must always be proprietary medicinal products and so come under the regulation of Directive 2001/83/EC. Various European countries, such as Belgium, the Netherlands, the United Kingdom and Germany, recognizing the importance of antisepsis of the skin in ensuring the safety of patients, have even anticipated these indications by the ECHA by incorporating the antiseptics used on the skin before a surgical intervention within the field of proprietary medicinal products [1].
In accordance with the current regulations, in Italy, antiseptics intended for use on injured skin and mucous membranes must be registered as "proprietary medicinal products" and, as such, must comply with Legislative Decree no. 219/2006 and subsequent amendments and supplements (implementing Directive 83/2001) and are subject to the respective production controls and ministerial authorization for release onto the market (Marketing Authorization). Antiseptics to be used on intact skin (e.g. hand washing by personnel and preparing a surgical site) and disinfectants for environmental use may, on the other hand, be registered with the Health Ministry as "medical-surgical aids". Finally, disinfectants for medical devices and/or equipment are registered as "medical devices" and must report the respective marking indicating conformity with EEC Directive no. 93/42 and subsequent amendments and supplements (from 26 May 2020, this will be repealed by the Medical Devices Regulation - MDR – EU 2017/745).

However, fundamental differences exist between a medicine and a biocide involving various aspects [2]:

MEDICINES		BIOCIDES
▪ Clinical studies are conducted in a regulated way in healthy subjects and in patients. ▪ The effectiveness, safety and quality of every product are tested by a competent authority. ▪ The evidence must emerge during the registration process, considering the benefits and potential risks of the product. ▪ The EMA's scientific guidelines on the clinical effectiveness and safety of medicines for human use help applicants prepare applications for marketing authorization.	**EVIDENCE OF QUALITY, SAFETY AND EFFECTIVENESS**	▪ Experiments or tests for the purpose of research or development of an unauthorized biocide or an unapproved active substance intended solely for use in a biocide are conducted according to the conditions established in the BPR (Biocides Regulation).
▪ The law requires that medicinal products for human use manufactured or imported in the EU comply with the guidelines on good manufacturing practice (GMP). ▪ Rigorous legislative prerequisites regarding the quality management system and on the pharmaceutical quality assurance system, etc. ▪ Production of medicinal products subject to constant official supervision and a pharmacovigilance system. ▪ Production of sterile products is subject to special prerequisites that minimize the risks of microbiological contamination.	**MANUFACTURE AND STERILITY**	▪ No specific prerequisite for the manufacturing process. ▪ Sterility is not required, nor any microbiological control
▪ Suppliers are subject to control and audit in accordance with GMP to check compliance with the specifications of the raw material provided and compliance with GMP. ▪ Suppliers are defined in the register and subject to approval and control in the case of changes and must indicate any modification to the competent authority and the clients who buy the product.	**DISTRIBUTION CHAIN**	▪ No control of raw material suppliers is required. ▪ For co-formulants, there is no specific indication and any co-formulant can be used, however for the active substance, procurement is required from the suppliers listed in article 95 of the BPR.
▪ The Community Register lists all the medicinal products for human and veterinary use and orphan medicines that have received marketing authorization through the centralized procedure. ▪ Some member states have instituted authorized medicinal product registers at the national level.	**REGISTER**	▪ Register of the Union for biocidal products.

Amended by [2].

For cutaneous antisepsis, the sterility of the solution is an important aspect to be considered.
In the EU, the Directive on Medicinal Products and the existing provisions on sterilization ensure the sterility of all medicinal products [3].
However, in non-sterile antiseptic solutions, contamination with some bacteria or spores can occur during the production process (intrinsic contamination) as fully documented in the literature [4-8].
In 2007, in the United States alone, more than 40 epidemics and pseudo-epidemics were reported due to contaminated antiseptics [8]. In Spain, batches of antiseptic solutions (classified as biocides) had to be withdrawn from the market because they were contaminated [8].

Implications

The use of biocides for medical purposes not only contradicts the aims of the provision on biocidal and medicinal products but also raises concerns from other points of view: patient and operator safety, environmental pollution and antimicrobial resistance.

PATIENT SAFETY

Biocides and medicines are subject to different regulatory pathways that have different standards in terms of safety, effectiveness and quality. It follows that using biocidal products as medicines (biocides do not have marketing authorization in accordance with the stringent rules of medicinal products) can put the patient's safety in jeopardy.
As made clear by the Medicines and Healthcare Products Regulatory Agency (MHRA), there are risks for health associated with the practice, and "using the suitably authorised product for the specific use envisaged, in accordance with the manufacturer's instructions for use, is the best way of minimising the danger" [9]. Studies have shown that biocides can have toxic and carcinogenic properties and harmful effects on the endocrine system [10].

ANTIMICROBIAL RESISTANCE

The previous Scientific Committee of the European Commission on emerging and recently identified risks for health (European Commission Scientific Committee on Emerging and Newly Identified Health Risks, SCENIHR) emphasized that, in order to preserve the role of biocides in the control of infections and hygiene, it is vital to prevent the emergence of bacterial resistance and cross-resistance through their appropriate and prudent use [11]. They added: "The need must be emphasised for the adequate use of disinfectants and antiseptics and health operators must be instructed to abide by clear and agreed policies and practices, avoiding the incorrect and unnecessary use of biocides" [11].
In other words: in the specific case of cutaneous antisepsis before medical treatments, the use of biocides must be limited to those cases for which they are strictly necessary and it is not possible to use a more suitable alternative, such as a medicine.
Moreover, as explained in the introductory section of this document, the improper use of antibiotics after blood cultures with false-positive outcomes not only exposes patients to the risk of serious adverse events without clinical benefit but also contributes to increased antimicrobial resistance.

OCCUPATIONAL SAFETY

Health operators can be directly exposed to biocides (primary exposure; i.e. the worker/operator uses a biocide on their skin) or indirectly (secondary exposure, i.e. after the effective use or application of biocidal products on the patient's skin). As mentioned earlier, biocides can have toxic and carcinogenic properties or harmful effects on the endocrine system that, especially in the case of the operators, may not be detectable.
In accordance with the Directive on Carcinogens and Mutagens 2004/37/EC, the employer must ensure that the risk to the health and safety of workers from hazardous substances is eliminated or reduced to a minimum (first level of the hierarchy of risk control). To fulfil this obligation, the employer's first priority is to replace or eliminate the risk of biocides, which can be implemented by using alternative disinfectants or replacing them with procedures, substances, preparations or less hazardous products.
Although various European and national guidelines exist that give instructions on the protection of operators during disinfection procedures in the health sector, the EU does not have specific harmonized guidelines on the safe use of biocides in the health sector.
The guidelines of the Directorate-General on Employment of the European Commission provide a general description of good practice regarding safe work in disinfection activities [12] that does not cover biocides and their use in the health sector.

ENVIRONMENTAL IMPACT

The use of biocides can also have significant environmental impact. In the health sector, the disposal of unused biocides or the residue of used biocides must be managed with extreme prudence in order to avoid serious environmental and potentially long-lasting damage.

For these reasons, a harmonized approach would be appropriate in the EU on the classification of cutaneous disinfectants before a surgical intervention or a medical procedure.

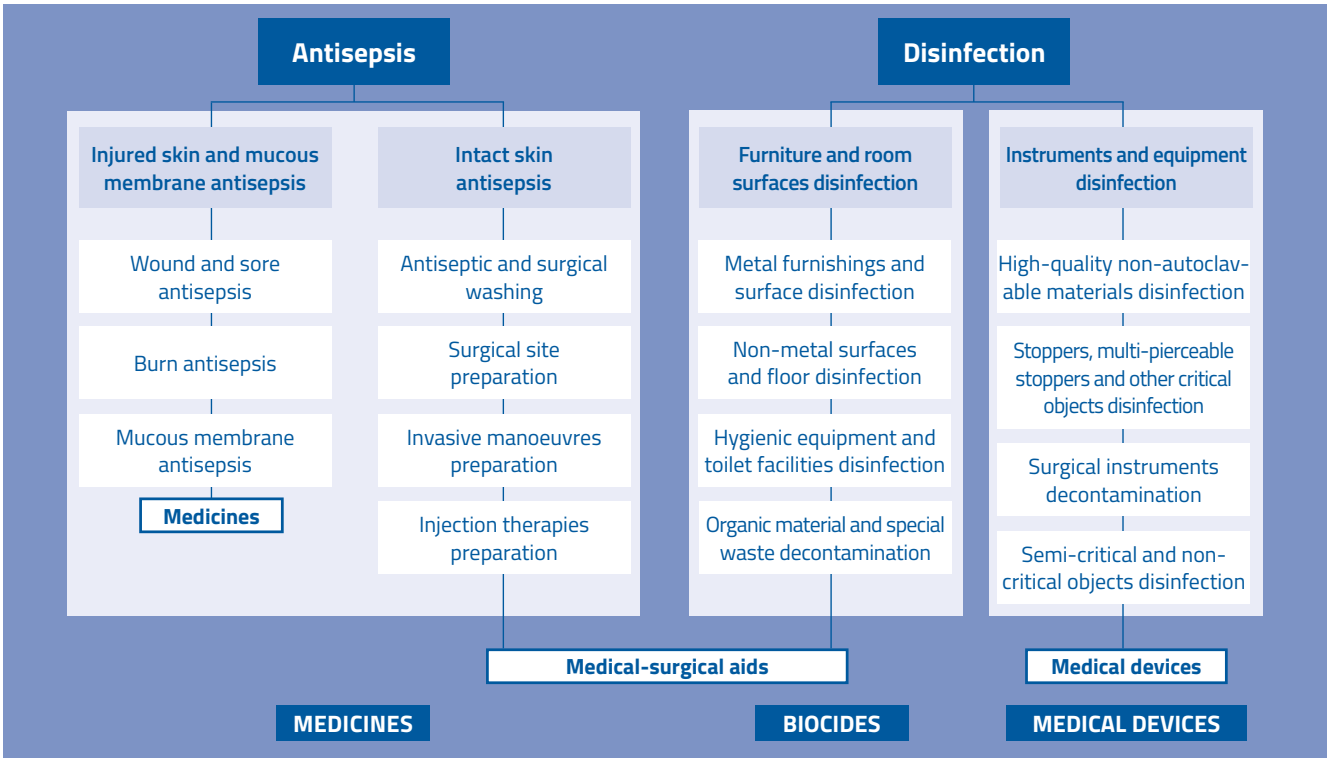
Regulatory aspects of the use of antiseptics and disinfectants

Implementation of Directive 98/8/EC regarding marketing authorization of biocidal products, implemented in Italy under Legislative Decree no. 174/2000 and later replaced by the regulation directly applicable in all member states (Regulation 528/2012, in force since September 2013), will in the near future lead to standardization of the authorization procedures for these products within the European Union. In Italy, the transition involves, in particular, current medical-surgical aids (now governed by Presidential Decree no. 392 of 6 October 1998) and numerous products that, although they have an intended biocidal use, are currently on the market without marketing authorization.

In this transition phase, a debate is underway within the institutions on the advisability of taking a position in line with that of other European countries with regard to the use of disinfectants and antiseptics for cutaneous antisepsis of the patient. Indeed, moving away from the Italian situation, in various European countries such as the United Kingdom, Germany, France, Belgium and the Netherlands, the regulations on disinfectants and antiseptics specify that, for surgical antisepsis of patients, even on intact skin, a product classified as a medicine must be used to ensure the safety, quality, sterility, tracking and traceability (see box on the following page) of the product used (Figure).

This concept also covers the so-called borderline products, that is, those that, by their nature, do not clearly belong to a specific sector with a unique regulatory qualification: chlorhexidine is a typical example.

Figure. Antiseptics and disinfectants based on the regulations on biocides (amended by [13])



TRACKING AND TRACEABILITY OF PRODUCTS

Tracking means the possibility of identifying and following a product through all phases of production, transformation and distribution up to the time of its use; it can be implemented at 2 different levels: tracking the batch and the individual unit.

Traceability, on the other hand, is the possibility of retracing the history of the product by backtracking through the process.

Tracking is a control activity instituted by the Health Ministry and the Italian Medicines Agency as a measure to protect public health, as a guarantee of integrity and prescriptive appropriateness of products for human use and aimed at combatting actions of counterfeiting, fraud and illegal trafficking. Tracking and traceability enable the correlation of a health product with the patient, as a guarantee of a high level of safety [14].

Article 40 of Law no. 39 of 1 March 2002 laid down the establishment of a central database at the Health Ministry that registers the movements of the individual packages of proprietary medicinal products, unequivocally identified by a unique stamp, referred to in Health Ministry Decree of 30 May 2014, bearing the marketing authorization code and progressive numbering of the individual packages, to guarantee complete tracking and traceability.

With the Decree of 11 June 2010, a database was also established for monitoring medical devices but the traceability system, although similar to the one for medicines, does not include the tracking of individual packages because they are not identified with a numbered stamp.

Regulatory criteria for medical-surgical aids and biocides that guarantee they can be tracked do not exist.

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