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Diagnostic stewardship in blood cultures: A literature review and adaptation to the EU context

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TABLE OF CONTENT

1. SUMMARY

This systematic review, conducted under the EU-JAMRAI 2 initiative (WP7 on Infection Prevention and Control), synthesizes evidence on diagnostic stewardship for blood cultures to combat antimicrobial resistance (AMR) and healthcare-associated infections (HCAIs) across Europe. Focused on optimizing blood culture use in adults and children, the review evaluated guidelines, systematic reviews, and grey literature (2023–2025) to address:

- Clinical indications for blood cultures to improve diagnostic accuracy and reduce unnecessary testing.
- Best practices in collection, processing, and interpretation, including contamination management.
- Procedural standards for sampling, laboratory communication, and result interpretation.

The INESSS guideline served as the foundational reference, supplemented by targeted searches in Medline, Cochrane Library, and European grey literature.

The prescription of blood cultures often involves controversial and non-consensual decisions. Furthermore, the misuse or misinterpretation of blood cultures and their results can lead to incorrect treatments, inappropriate patient isolation, and suboptimal management strategies. These issues may result in prolonged hospital stays, adverse events, and increased use of antimicrobials, causing greater patient discomfort, and ultimately leading to higher antimicrobial resistance.

To address these challenges, comprehensive and up-to-date scientific evidence has been compiled into this final resource to inform clinical and policy decision-making.

2. INTRODUCTION

This document compiles the findings of a systematic review of clinical practice guidelines and relevant literature on diagnostic stewardship in blood culture practices. The review was conducted within the framework of the Infection Prevention and Control (IPC) Work Package (WP7) of the EU-JAMRAI 2 initiative—an EU Joint Action aimed at addressing antimicrobial resistance (AMR) and healthcare-associated infections (HCAIs) through coordinated One Health strategies across Member States.

The purpose of the review was to assess and synthesise existing guidance and recommendations related to the appropriate use of blood cultures in both adult and paediatric populations. Blood cultures are essential for detecting bloodstream infections and guiding appropriate antimicrobial therapy. However, a large proportion of cultures in clinical practice yield negative results or are compromised by contamination. These limitations highlight the need for stewardship interventions that promote appropriate ordering practices, improve sampling techniques, and reduce unnecessary testing.

The review placed particular emphasis on identifying best practices that support the rational use of blood cultures, including the timing, clinical indications, and techniques for sample collection. Existing resources, such as the *Guide and Standard: Judicious Blood Culture* developed by the Institut national d'excellence en santé et en services sociaux (INESSS), were used as foundational references. To broaden the evidence base and ensure applicability to the European context, the review also included additional national and international clinical guidelines, systematic reviews, and relevant grey literature.
In addition to published sources, the knowledge and expertise of European partners actively working in the field of infection prevention and diagnostic stewardship were essential to this review. Their contributions enriched the analysis with practical insights and contextual understanding, particularly in relation to the diversity of healthcare systems across the European Union.

The results of this review are intended to serve as a consolidated evidence base to support the expert consensus process led by the European Centre for Disease Prevention and Control (ECDC). The compiled evidence will contribute to the development of future recommendations on blood culture practices, as part of the forthcoming *EU infection prevention and control guidelines in human health*. These guidelines are being developed under the Council Recommendation on stepping up EU actions to combat AMR through a One Health approach (2023/C 220/01), in collaboration with the European Commission and the ECDC.

3. METHODOLOGY

3.1 Research questions

Questions were formulated according to the elements of the PIPOH model: **p**opulation, **i**nterventions of interest, **p**rofessionals for whom the work is intended, **o**utcomes and the environment and clinical context in which the interventions are applied (**h**ealth care setting).

Decision question

What are the good clinical practice guidelines for the judicious use of blood cultures to ensure diagnostic accuracy, reduce unnecessary testing, minimize contamination, and improve patient outcomes?

Clinical dimension

1. In which clinical situations should blood cultures be taken or repeated in adult and paediatric populations to improve diagnostic accuracy and patient outcomes?

2. What constitutes good clinical practice for performing blood cultures in patients with specific conditions to optimize diagnostic accuracy and clinical outcomes?

3. What are the best practice procedures for:

a. Collecting blood samples for blood cultures?

b. Transmitting accurate and complete information to medical laboratories when requesting blood cultures?

c. Interpreting and managing blood culture results, including the identification and handling of common contaminants, to improve patient care and outcomes?

3.2 Literature search strategies

As a first approach to the literature, an initial scoping review was conducted to identify existing guidelines, protocols and recommendations related to blood culture practices and clinical guidelines for infections that typically require blood cultures. This initial search yielded multiple clinical guidelines, among which the INESSS guide (*Guide and Standard: Judicious Blood Culture*) (Morrow & Clavet-Fournier, 2024) stood out as particularly comprehensive, as it encompassed 2 systematic reviews and compiled grey literature until 2023. Given that its methodology aligned with our approach and its evidence synthesis is up-to-date, it was decided to adopt this guide as the foundational reference for our project to prevent redundant work and duplication of efforts. The remaining guidelines identified in our scoping review were found to be less exhaustive than the INESSS guide; nevertheless, they were used as additional grey literature. The selection of keywords for this initial search was carried out with the guidance of experts in bibliographic research and scientific advisory support.

Subsequently, a second and more targeted search was conducted, following the 1st systematic review search strategy previously employed by the INESSS guide, that addressed clinical practice guidelines on blood cultures. The comprehensive search methodology used in INESSS's second systematic review, which focused on identifying clinical conditions that may have blood cultures as

a basic work-up, was not repeated for this diagnostic stewardship guideline, as such an extensive review was beyond the document's intended scope.

This final document of recommendations was focused on updating the clinical information in the INESSS recommendations document, incorporating new evidence and state-of-the-art topics published since the document's last release.

The systematic search was performed with the support of CatSalut and Universitat de Barcelona (UB) documentalists and included clinical practice guidelines, expert consensus documents, protocols, systematic reviews and other similar publications published between 2023 and 2025, and was performed on Medline (OVID) and Cochrane Library (Web). Other expert sources, including grey literature from European countries, were consulted: Websites of agencies involved in the evaluation of health and social care technologies and public health or regulatory agencies, as well as those international and governmental bodies. Finally, the following search methods were also used: Google search engine and search for references in bibliographies.

The methodological quality of the documents used in the INESSS guideline were not assessed as the quality of evidence of their recommendations was already evaluated using the AGREE-II Tool.

3.3 Literature selection criteria

Two scientific experts independently selected all publications identified by the bibliographic search based on their titles and abstracts. Later, the documents obtained from this first selection were revised again by reading the full length of the document. The screening of the documents was performed applying the established inclusion and exclusion criteria using Covidence tool (see table 1 below). Disagreements were resolved by the opinion of a third person.

In case of multiple publications, only the most recent version was retained for analysis.

Table 1. Inclusion and exclusion criteria for documents containing clinical information and recommendations.

Elements	Inclusion criteria
POPULATION	Children and adults in whom: • a blood culture is being considered • an indication targeted by the Advisory Committee is suspected
INTERVENTION	Blood culture
PROFESSIONALS INVOLVED	Health care professionals

PARAMETERS OF INTEREST (ASPECTS TO BE DOCUMENTED)	**Context in which blood cultures should be collected** (questions 1 and 2) o <u>Initial blood culture request:</u> ▪ When blood cultures should be requested. ▪ Clinical situations in which blood cultures are likely to be required as part of the basic work-up. ▪ Recommendations for blood collection in certain specific clinical situations. **How to collect blood samples (question 3a)** o <u>Timing of blood collection:</u> • In relation to signs and symptoms of bacterial infection. • Versus other blood tests. • In relation to antibiotic therapy. o <u>Sampling strategy:</u> • Number of sampling sites. • Volume of blood to be collected. o <u>Sampling:</u> • Choice of sampling site(s). • Skin preparation. • Blood culture bottle identification. • Storage of blood culture bottles until transport. **Information to be sent to the laboratory** (question 3b) **Post-extraction considerations** (question 2 and 3a) ○ Rapid tests vs blood cultures ○ Microbiology laboratory considerations **Interpretation and management of results, including information about contamination** (question 3c) o <u>Interpretation of results:</u> ▪ *Risk of false negative results:* Factors that increase the risk of false negative results. ▪ *Risk of false positive results:* Criteria for distinguishing true positives from false positives. ▪ *Bacterial species identified in blood cultures*: Common contaminants vs pathogenic species. • **Follow-up blood cultures** (question 1):

	o <u>When repeating blood cultures is recommended:</u> ▪ Information to support recommendations concerning the indications for requiring follow-up blood cultures in clinical situations for which blood cultures are recommended as part of the basic work-up. ▪ Information to support recommendations on the indications for follow up blood cultures in clinical situations, depending on the aetiology of the infection. ▪ Contamination rates considerations
CLINICAL CONTEXT	Hospital
TYPE OF PUBLICATION	Good clinical practice guides, expert consensus, guidelines, protocols, pathways, systematic reviews and meta-analysis
PUBLICATION YEAR	2023-2025
Exclusion criteria	
POPULATION	No exclusions will be made based on population
CLINICAL SETTING	Programmed non-complicated surgeries

3.4 Methodological quality assessment

The **AGREE II** tool (Appraisal of guidelines for research and evaluation) was used for quality assessment of the guides and recommendations found in the currents research. The domain we have focused on to evaluate the quality was Domain 3: Rigour of development. Guidelines were rated as high-quality when two independent reviewers assigned scores exceeding 70%, and medium-quality for scores above 50%. The documents were scored by two independent reviewers, and since no significant differences were found, involving a third expert was unnecessary.

For the quality evaluation of systematic review and meta-analysis articles **AMSTAR-II** tool (Assessing the Methodological Quality of Systematic Reviews) was used to evaluate the quality of the results. The documents were scored by two independent reviewers, and since no significant differences were found, involving a third expert was unnecessary.

3.5 Analysis and summary

An analysis was carried out by scientific experts to identify similarities and differences in the information gathered in shared excel document, which then were summarised in a narrative synthesis, taking into account the methodological quality of the documents and comparing the evidence to the recommendations of the INESSS reference document.

4. ARGUMENTS AND FINDINGS

4.1 General points

While the body of evidence we compiled largely aligns with the INESSS reference guide, our systematic review yielded several important refinements and expansions to the existing recommendations. Notably, we identified emerging evidence that:

Advances in Diagnostic Technologies:

- Provides new insights into the clinical applications and limitations of rapid molecular testing
- Clarifies their role as adjuncts to conventional blood cultures

Contemporary Challenges:

- Addresses previously underexplored aspects of diagnostic stewardship
- Offers updated perspectives on optimal implementation strategies

Evidence Gaps:

- Highlights areas where new research has modified previous understandings
- Identifies persistent clinical uncertainties requiring further investigation

4.2 Initial blood culture request

Evidence in reference document:

In the INESSS reference document, only one of the systematically reviewed publications provided general recommendations on the decision to use blood cultures. According to this document, the choice to perform blood cultures should be based either on the pre-test probability of bacteraemia or on the potential benefits of the result (e.g., additional microbiological information or impact on clinical management).

The remaining documents focused on specific clinical conditions where blood cultures may or may not be included in the initial workup. These conditions include **septic arthritis, meningitis, sepsis and the presence of neutropenic fever in patients undergoing chemotherapy.** Additionally, certain clinical factors were noted to increase the likelihood of bacteraemia, such as the presence of severe chills or comorbidities, and the severity of systemic involvement.

Beyond the reviewed documents, clinicians identified other high-risk situations that could justify blood culture testing if any symptoms compatible with bacteraemia or sepsis are present:

- sickle-cell anemia, since sufferers are instructed to go to emergency as soon as they develop a fever
- being pregnant, since at the end of pregnancy there is a certain degree of immunosuppression, and in particular a risk of listeriosis
- the presence of co-morbidities, which can alter the body's ability to function properly

Evidence compilation update:

New evidence found in this review updates and complements the INESSS reference document, focusing on clinical criteria to guide blood culture request:

When to obtain blood cultures

- Blood cultures should be obtained based on **clinical suspicion of sepsis or bacteraemia**, especially in patients requiring hospitalization or close monitoring (Bonomo et al., 2024; Fontana et al., 2023)
- Additional specific scenarios where blood cultures are recommended:
    - High regional prevalence of **resistance** to empiric antimicrobials for **intra-abdominal infections** (Schoffelen et al., 2024).
    - Recent (within 90 days) **colonization or infection with drug-resistant microorganisms** (Schoffelen et al., 2024).
    - **Healthcare-associated infections** (Schoffelen et al., 2024)
- Blood cultures are advised in cases with **laboratory abnormalities** or infection signs such as leucopenia, leucocytosis, thrombocytopenia (not related to haematological treatment), focal infection signs, **suspected endocarditis, or catheter-related infections** (Rodríguez Díaz et al., 2017).
- Consider blood cultures in patients with **fever of unkown origin with risk of sudden deterioration,** specially in patients over 65 years and chronically ill individuals, (Clinical Excellence Commission, 2024a).
- Consider obtaining blood cultures in the following conditions: Infections involving **implanted medical devices**, such as vascular catheter or ventriculo-atrial shunts, **febrile** patients with **anatomical or functional hyposplenism**, i**mmunocompromised** patients presenting with new fever or signs of infection without an evident source, **acute pyelonephritis** requiring hospitalization, **severe community-acquired pneumonia** at the time of hospital admission, **cellulitis requiring admission**, particularly when *group A. Streptococcus* infection is suspected, **prosthetic infections** with signs of sepsis, and **fever in returned travelers** where **typhoid fever** is clinically suspected based on travel history.

There are specific recommendations on whether or not to take blood cultures in **Emergency Departments (EDs)**, based on certain clinical criteria. There are two guides that specifically address these conditions, one of high quality (Schoffelen et al., 2024), the other of medium quality (Gamazo Del Río et al., 2025).

Community acquired pneumonia (CAP) - Emergency Department considerations

It **is recommended** to obtain blood cultures under the following conditions:

- In patients admitted with severe CAP, e.g. patients with PSI score IV or V or with indications for ICU admission.
- In patients admitted with CAP and risk factors for or initiated on therapy for unusual or resistant pathogens.
- In patients admitted with CAP and immunocompromised state (Schoffelen et al., 2024).
- In patient where hospitalisation is indicated or requiring empirical treatment for methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa*, as well as those who have had previous infections with these pathogens or hospitalisation in the last 90 days or other risk factors for resistant pathogens (Gamazo Del Río et al., 2025).

It is suggested **against** obtaining blood cultures routinely in patients presenting to the ED with a diagnosis of non-severe CAP (Schoffelen et al., 2024).

Urinary tract infection (UTI) with systemic symptoms - Emergency Department considerations

It **is recommended** to obtain blood cultures under the following conditions:

- In patients presenting to the ED with UTI with systemic symptoms and antibiotic pretreatment.
- In patients presenting to the ED with a chronic indwelling catheter and UTI with systemic symptoms.
- In immunocompromised patients presenting to the ED with UTI with systemic symptoms (Schoffelen et al., 2024).
- In acute pyelonephritis (APN) admitted to hospital and complicated APN, as well as in patients with renal insufficiency, immunosuppression, on haemodialysis or with risk factors for resistant pathogens (Gamazo Del Río et al., 2025).

It is suggested **against** obtaining blood cultures routinely in patients presenting to the ED with UTI with systemic symptoms (except with signs of bacteriemia or sepsis) without anatomical abnormalities of the urinary tract in whom a good-quality urine sample for culture is available (Schoffelen et al., 2024).

Skin and soft tissue infections - Emergency Department considerations

It **is recommended** to obtain blood cultures under the following conditions:

- Immunocompromised patients presenting to the ED with cellulitis/erysipelas.
- In patients presenting to the ED with cellulitis/erysipelas in clinical situations associated with high risk of non-standard pathogens.
- In patients presenting to the ED with cellulitis/erysipelas who have an intravascular prosthesis, a pacemaker or a valvular prosthesis (Schoffelen et al., 2024).
- In the case of cellulitis if the patient has immunosuppression or risk factors for resistant pathogens or other conditions (high comorbidity, diabetes, patients over 65 years) that indicate hospital admission. In addition, in all cases of suspected or confirmed pyomyositis, deep or necrotising infections (Gamazo Del Río et al., 2025).
- Patients with severe skin or soft tissue infections requiring hospitalization.

It is suggested **against** routinely obtaining blood cultures in patients presenting to the ED with cellulitis/erysipelas without other signs or clinical conditions (Schoffelen et al., 2024).

When is generally NOT recommended to obtain blood cultures (always considering that clinical judgment must prevail):
Blood cultures are generally **not recommended** in scenarios with low diagnostic yield, including but not limited to the following:

- **Isolated abnormalities without other signs of infection:**
  - Isolated fever or isolated leucocytosis (Clinical Excellence Commission, 2024b; Rodríguez Díaz et al., 2017).
- **Repeat cultures without new clinical changes:**
  - Persistent fever/leucocytosis after negative cultures within 72 hours.
  - To document clearance of bacteraemia except for certain pathogens or when endovascular infection or persistent bacteraemia is suspected (Rodríguez Díaz et al., 2017).
- **Low-risk contamination or screening:**
  - To rule out contamination in immunocompetent patients without prosthetic implants.
  - Routine surveillance cultures without suspicion of bacteraemia (e.g., prior to TPN, central line placement, sedation weans, ECMO, CRRT) (Rodríguez Díaz et al., 2017).
- **Specific clinical conditions with low yield:**
  - Community-acquired pneumonia not requiring ICU care.
  - Cellulitis not requiring hospitalization.

- Fever within 48 hours post-surgery.
- Lower urinary tract infections (e.g., cystitis, prostatitis) where urine culture is preferred.
- Ventilator-associated pneumonia, hospital-acquired or aspiration pneumonia.
- COPD exacerbations (Clinical Excellence Commission, 2024b; Rodríguez Díaz et al., 2017).

Paediatric:

The blood culture extractions are indicated in paediatric patients with a sudden decline, since in this populations the typical signs and symptoms of bacteraemia may not be present. Blood culture should be complemented with samples from other sites to try to determine the focus of the process (Rodríguez Díaz et al., 2017).

The recommendations for blood culture use in paediatric patients outlined in the CEC (Clinical Excellence Commission, 2024c) low-quality guideline do not differ substantially from those applied to adults, as the same high-risk conditions, such as sepsis, bacterial meningitis, endovascular infections, and deep-seated infections, are similarly emphasized. However, this paediatric guidance adds clinically relevant nuances by explicitly highlighting the need to consider blood cultures in immunocompromised children presenting with fever or signs of infection without an evident source, and in those with splenectomy or functional hyposplenism. These distinctions underscore the heightened vulnerability of paediatric patients in certain scenarios and support a more proactive diagnostic approach in this population.

Key Recommendations Summary

1. Clinical Indications for Blood Cultures

- Strongly Recommended:
  - Sepsis or systemic infection suspicion.
  - High-risk conditions:
    - Meningitis
    - Septic arthritis
    - Neutropenic fever (chemotherapy patients)
    - Implanted device infections (e.g., catheters, shunts)
    - Immunocompromised patients with fever of unknown origin
    - Sickle-cell anemia with fever
    - Pregnancy (3rd trimester, listeriosis risk)
- Emergency Department (ED) considerations:
  - Community-Acquired Pneumonia (CAP): Only if severe (ICU admission, resistant pathogens)
  - UTI with systemic symptoms: If immunocompromised, indwelling catheter, or pyelonephritis

- Skin/soft tissue infections: If immunocompromised, prosthetic devices, or necrotizing infection

2. When is generally NOT recommended to obtain blood cultures (always considering that clinical judgment must prevail):

- Low-yield scenarios:
  - Isolated fever or leukocytosis without infection signs
  - Non-severe CAP or cellulitis not requiring hospitalization
  - Lower UTIs (prefer urine cultures)
  - Routine surveillance (e.g., pre-procedure)

3. Best Practices for Collection & Interpretation

- Timing:
  - Early testing at first suspicion of bacteraemia
  - Avoid repeats before 72h of targeted therapy (except for S. aureus, endocarditis, candidemia)
- Clinical Correlation:
  - Do not dismiss contaminants (e.g., coagulase-negative staphylococci) without assessing patient risk factors
  - Verify prior cultures in transferred patients to avoid unnecessary repeats

4. Paediatric Considerations

Includes standard adult indications (e.g. sepsis, deep infections), with additional emphasis on:

- Acute clinical decline (atypical presentations)
- Immunocompromised children or those with functional hyposplenism
- Complement with samples from other sites to identify infection focus

5. Key Updates from New Evidence

- Resistance-driven testing:
  - Obtain cultures if high regional AMR prevalence or prior resistant infections
- ED-specific protocols:
  - Avoid routine cultures in non-severe infections (e.g., uncomplicated cellulitis)

Takeaway:

- Targeted use improves diagnostic yield and antimicrobial stewardship.
- Clinical judgment overrides protocolized testing in ambiguous cases.

4.3 Blood sampling

4.3.1 Timing of blood sampling

Evidence in reference document:

The INESSS reference document stated that timing has little impact on blood cultures results, implying that waiting for a peak in symptoms to occur before sampling is not recommended. It was also stated that blood cultures should be drawn prior to conducting other tests, as those tests might influence the blood culture outcomes. Furthermore, all the reviewed documents emphasized that initiating the antibiotic therapy before taking blood samples can compromise blood culture results and therefore recommend collecting blood samples first, whenever the patient's clinical condition permits.
The maximum recommended time between clinical examination and initiation of antibiotic therapy varies depending on the clinical situation, from 6 hours in the least urgent situations to 1 hour in urgent clinical conditions. However, in patients suspected of having meningitis, sepsis, or septic shock, antibiotics should be administered within 1 hour of the clinical assessment.

Evidence compilation update:

A medium-quality guideline (Suleyman, 2024) and low-quality document ((Rodríguez Díaz et al., 2017) both consistently emphasize the importance of collecting blood cultures prior to initiating antimicrobial therapy, in line with the INESSS recommendations. The Suleyman (Suleyman et al., 2024) guide, along with the guide of the Associazione Microbiologi Clinici Italiani (AMCLI) (Fontana et al., 2023), further specified that multiple cultures should be drawn consecutively without time intervals, as the timing of fever does not influence culture yield, an approach also endorsed by INESSS.

In a low quality guideline, it was discussed that if it’s not possible to draw the blood for the blood cultures before starting the antibiotic therapy, the blood should be extracted when the concentration of antibiotics is at its lowest. It was also recommended that, in case of acute infection, the blood extraction should be performed when chills appear (Rodríguez Díaz et al., 2017).

High-quality evidence (Bonomo et al., 2024) establishes that collection should never be delayed to assess hemodynamic response to fluids or whether delirium is new-onset.

Paediatric:

A recent study analysing surveys of paediatric physicians found that half reported routinely obtaining blood cultures even when bacteraemia was not clinically suspected, most commonly when blood was already being drawn for other purposes (Duguid et al., 2025).

Key Recommendations Summary

Timing of Blood Cultures:

- Draw **as soon as infection is suspected**
- Collect **before antibiotics** (when possible) to avoid false negatives.
- Perform **before other tests** to prevent interference.

Antibiotic Timing:

- Start antibiotics **within 1 hour** for sepsis, meningitis, or septic shock.
- **Within 6 hours** for less urgent cases.

Special Case:

- If antibiotics **must start first**, draw cultures at **lowest antibiotic concentration** (e.g., just before next dose).

Avoid Delays:

- Never wait for **hemodynamic stability** or delirium assessment.

4.3.2 Strategy of sampling

4.3.2.1 Number of sampling sites

Evidence in reference document:

The INESSS reference document highlights that 5 of 6 reviewed guidelines recommend obtaining blood cultures from a minimum of two separate sites in both adults and children. The exception is neonates, for whom a single sampling site is generally preferred (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; Doern et al., 2019; OPTMQ, 2018). However, one guideline (De Plato et al., 2019) advocates for single-site sampling to minimize the risk of contamination.
Further research (Andersson Norlén et al., 2023; Mahieu et al., 2023; Ekwall-Larson et al., 2022; Yu et al., 2020; Lamy et al., 2016; Dargère et al., 2014; Lamy et al., 2002; Arendrup et al., 1996) compared the single-puncture (four bottles) versus multiple-puncture (two bottles per puncture) techniques. These studies consistently concluded that the single-puncture approach yields non-inferior diagnostic performance and is associated with lower contamination rates. Additionally,

expert panels noted that single-site collection enhances patient comfort and nursing efficiency, as it reduces procedure time. Several documents (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; De Plato et al., 2019; OPTMQ, 2018) also addressed the optimal time interval between blood culture collections when using multiple sites. Recommendations varied depending on clinical context: shorter intervals (10–60 minutes) are advised in urgent cases, whereas longer intervals may be acceptable for clinically stable patients.

Evidence compilation update:

As a previous consideration, we studied the number of sets to be collected when performing a blood culture (Table 2).

Table 2. Recommendation of set collection.

Scenario	Recommendation	Evidence Quality
Adult patient	2-3 sets	High (Bonomo, 2024), Medium (Suleyman, 2024; Fontana, 2024), Low (Bunn, 2025; NHS, 2023)
Endocarditis / prosthetic device infection / catheter infection	4 sets	Low (Rodríguez Díaz, 2017)
Pediatric patient	2 sets (or 1 aerobic if ≤1 kg)	Medium (Suleyman, 2024), Low (CEC, 2024c)

Only one medium-quality guideline (Fontana et al., 2023) discussed the topic regarding the number of sampling sites. In the guideline it was concluded that the ‘single sample strategy’ offers some advantages over the current standard based on the 'multi sample strategy,' especially when a dedicated staff team is available and generally in intensive care units. The 'multi sample strategy' is used in specific circumstances, not only related to the type of patient but more often to the suspected infection, aligning with the INESSSS recommendations. Contrarian to this evidence, a high-quality guideline (Bonomo et al., 2024) emphasizes the importance of using distinct venipuncture sites for each culture set.

Additional evidence from a low-quality guideline (Rodríguez Díaz et al., 2017) explicitly contraindicates blood culture collection from intravascular devices and diverges from the INESSS guidance by recommending that, in cases requiring multiple draws, samples should be collected 10 to 30 minutes apart, with the possibility of shortening this interval in urgent situations.

Paediatric:

Regarding paediatric sampling sites, only one low quality guideline (Clinical Excellence Commission, 2024c) provided specific guidance, recommending that clinicians use separate peripheral sites for any additional required blood culture sets.

Key Recommendations Summary
Blood Culture Collection Methods

1. Preferred Method: Single Puncture (4 bottles) - Recommended for most cases (adults and children)
    a. Advantages:
        i. Equal diagnostic yield than multiple punctures
        ii. Lower contamination risk
        iii. Improved patient comfort and nursing efficiency
        iv. No delay between samples
2. Multiple Punctures (2 bottles per puncture) - Only for specific cases:
        i. Suspected endovascular infections (e.g., endocarditis, catheter infections).

Number of Sets

- Adults: 2–3 sets (standard cases).
- Endocarditis/prosthetic device infections: 4 sets.
- Paediatrics: 2 sets (1 aerobic if ≤1 kg).

Neonates

- Single-site sampling preferred.

4.3.2.2 Volume of blood to be collected

Evidence in reference document:

The INESSS reference document underscores that blood volume collected is the most critical determinant of blood culture sensitivity. Both underfilling and overfilling culture bottles can compromise diagnostic accuracy. For adults, the recommended volume is 8–10 mL per bottle, while for paediatric patients, it is typically 4 mL (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; Gorski et al., 2021; De Plato et al., 2019; Doern et al., 2019; OPTMQ, 2018). Some guidelines (e.g., Gorski et al., 2021; De Plato et al., 2019) set a lower threshold of 5 mL for adult bottles, and Miller et al. (2024) suggests a minimum of 2 mL for paediatric bottles.

Several sources recommend weight-based guidance for blood volume collection in children. Four key documents advise that the volume drawn should not exceed 1% of total blood volume in neonates and young children (UKHSA, 2023; CLSI, 2022; Gorski et al., 2021; OPTMQ, 2018). However, Miller et al. (2024) offers a chart allowing up to 4% of total blood volume in selected clinical situations, thereby providing flexibility.

In paediatric patients, limited circulating blood volume often restricts the quantity of blood that can be drawn. As a result, a single aerobic paediatric blood culture bottle is frequently used. Nonetheless, anaerobic bottles may be appropriate in specific clinical contexts, including:

- Intra-abdominal infection
- Necrotizing enterocolitis
- Head and neck infection
- Immunosuppression
- Perianal or gynaecologic infections
- Bite wounds
- Neonates born to mothers with chorioamnionitis

Depending on the child's weight, it may be appropriate to collect additional paediatric bottles, or in some cases, adult-sized bottles. When collecting ≥5 mL, the volume should be equally divided between two blood culture bottles to optimize yield and maintain bottle function.

Evidence compilation update:

Four low-quality documents (Bunn & Cornish, 2025; Clinical Excellence Commission, 2024a; NHS England, 2023; Rodríguez Díaz et al., 2017) and one medium-quality guideline (Fontana et al., 2023) recommend collecting 20–30 mL of blood per culture set, with weight-based adjustments when appropriate, consistent with the standards outlined in the INESSS guideline. Additionally, a medium-quality guideline (Suleyman et al., 2024) emphasizes the importance of filling each bottle to the manufacturer's recommended optimal volume, while explicitly warning against overfilling beyond the maximum capacity, as this may compromise culture performance.

Paediatric:

Current evidence consistently supports weight-based volume adjustments for paediatric blood cultures, in line with the INESSS guidelines. This approach is endorsed across documents of varying methodological quality (Bonomo et al., 2024; Clinical Excellence Commission, 2024c; Suleyman et al., 2024), all of which emphasize its importance for optimizing diagnostic yield. Notably, these sources highlight the preferential use of aerobic bottles when blood volume limitations allow for only a single-bottle collection, particularly in younger children.

For neonatal and infant populations, the low-quality guideline from SEIMC (Rodríguez Díaz et al., 2017) and a physician survey (Duguid et al., 2025) specify a minimum volume of 1 mL, acknowledging the challenges of small volume draws in this vulnerable group.

The low-quality guideline from CEC (Clinical Excellence Commission, 2024c), provides additional granularity, proposing an age-based calculation where the minimum volume (in mL) equals the child's age (e.g., 2 mL for a 2-year-old), with an upper limit of 10 mL to match adult standards.

Importantly, this guideline introduces a critical safety parameter: total collected volume should never exceed 4% of the child's total blood volume, a safeguard against iatrogenic anaemia.

Key Recommendations Summary

Adults:

- Total per set: 20–30 mL (divided between bottles).

Pediatrics:

- Weight-based calculation: Do not exceed 1–4% of total blood volume.
- Minimum volumes:
    i. Neonates/infants: ≥1 mL
    ii. Children: Weight-based, max 10 mL
- Prioritize aerobic bottles (single-bottle use if limited volume).
- Anaerobic bottles for specific cases (e.g., intra-abdominal infections, immunosuppression).

General Principles:

- Avoid over/underfilling bottles (follow manufacturer guidelines).
- Collect aerobic bottles first during venipuncture.

4.3.3 Taking samples

4.3.3.1 Choice of sampling site(s)

Evidence in reference document:

The INESSS reference guideline recommends peripheral venous sampling as the preferred method for obtaining blood cultures, noting that while arterial sampling is technically feasible, it offers limited diagnostic advantages. Moreover, sampling from vascular access devices (VADs) is associated with an increased risk of contamination (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; De Plato et al., 2019).

The expert panel further clarified that, in both adults and children, freshly inserted VADs may be acceptable for blood culture collection. However, specific considerations apply in paediatric and neonatal populations, where preserving venous access is critical. In these settings, particularly in neonatology, central and peripheral catheters are frequently used. The expert panel agreed that blood should not be drawn from a peripheral catheter unless it is freshly placed. In contrast, central lines may be used, especially when venous access is limited, as is often the case in infants and young children. When using a central line, the panel emphasized the importance of simultaneous peripheral sampling, when feasible, to improve diagnostic interpretation and distinguish contamination from true bacteraemia

Evidence compilation update:

Three documents addressed the issue of optimal sampling site selection for blood cultures.

A low-quality guideline (Clinical Excellence Commission, 2024a) strongly recommends prioritizing peripheral venipuncture over line-based collection. When a catheter draw is unavoidable, it should be paired with a peripheral sample to facilitate interpretation and help distinguish contamination from true infection.

Additionally, a high-quality systematic review (Sautter et al., 2024) emphasizes the importance of establishing institutional protocols to ensure strict adherence to sterile technique during peripheral venipuncture, thereby minimizing contamination risk.

Finally, a medium-quality guideline (Suleyman et al., 2024) provides a specific recommendation for neutropenic patients, advising against collecting multiple blood culture sets from each port of a single central line, due to increased risk of contamination and potential diagnostic inaccuracy.

Paediatric:

No new evidence was found regarding this topic in paediatric population.

Key Recommendations Summary

Preferred Sampling Method

- Peripheral venipuncture is the gold standard for blood cultures due to lower contamination risk.
- Avoid arterial sampling (minimal diagnostic benefit).

Vascular Access Devices (VADs)

- Freshly installed peripheral catheters (including PICC lines) may be used if:
  - Strict no-touch antiseptic technique is followed.
- Central lines:
  - Only use if:
    - VAD infection is suspected.
    - Peripheral access is impossible (e.g., paediatric/neonatal cases).
  - Always pair with a peripheral sample for comparison when diagnosing VAD infections.
  - Interpret results cautiously (higher contamination risk).

Special Populations

- Neutropenic patients: Avoid multiple cultures from a single line port.
- Paediatrics/neonates: Central lines may be used if peripheral access is limited, but peripheral sampling remains preferred.

Procedural Best Practices

- Institutional protocols should ensure sterile technique during peripheral draws.
- For VAD infections: Collect one set from the line + one peripheral set for comparison.

4.3.3.2 Preparing the skin

Evidence in reference document:

The INESSS reference documents consistently emphasize that proper skin antisepsis is critical for minimizing contamination risk in blood culture collection. While chlorhexidine-based preparation remains the gold standard for most patients, specific populations require modified protocols due to skin fragility or allergy concerns (Miller et al., 2024; CLSI, 2022; De Plato et al., 2019; Doern et al., 2019; OPTMQ, 2018). Inadequate skin preparation can significantly compromise diagnostic accuracy, potentially leading to inappropriate clinical management, delayed treatment, and inefficient resource utilization.

Evidence compilation update:

Five documents, including guidelines and a systematic review, addressed skin antisepsis for blood culture collection. Four publications across all evidence levels (Clinical Excellence Commission, 2024a; Fontana et al., 2023; Rodríguez Díaz et al., 2017; Sautter et al., 2024; Suleyman et al., 2024) recommended using 2% chlorhexidine in 70% alcohol, consistent with INESSS guidelines.
Additionally, two low-quality documents (Clinical Excellence Commission, 2024a, 2024c) suggested 70% isopropyl alcohol as an alternative antiseptic option.

Paediatric:

While 2% alcoholic chlorhexidine is recommended for paediatric patients by two low-quality guidelines (Clinical Excellence Commission, 2024c; Rodríguez Díaz et al., 2017), both sources concur with INESSS in advising against its use in neonates under 2 months. This precaution reflects the burn risk associated with chlorhexidine applications on non-keratinized skin, favouring aqueous solutions instead.

Key Recommendations Summary

- Dual antisepsis is required:
  - First cleanse: General skin cleaning.
  - Second cleanse: 2% chlorhexidine in 70% alcohol (gold standard).

Special Populations

- Neonates (<2 months):
  - Avoid alcoholic solutions on non-keratinized skin; preferably use aqueous solutions instead.
- Chlorhexidine allergy:
  - Replace with two sanitizations using 70% alcohol (three total if including initial cleaning).

Alternative Options

- 70% isopropyl alcohol is an acceptable alternative.

4.3.3.3 Identification of the blood cultures bottles

Evidence in reference document:

The INESSS reference guide, along with several reviewed documents, emphasizes the importance of ensuring that information recorded on blood culture bottles is accurate and complete to support appropriate interpretation of results (Miller et al., 2024; CLSI, 2022; Gorski et al., 2021; De Plato et al., 2019; OPTMQ, 2018). These documents provide specific examples of essential data to be included, such as patient identifiers, date and time of collection, sampling site, and collector's identity.

Furthermore, two sources (CLSI, 2022; Gorski et al., 2021) highlight the need for this information to be recorded at the time of blood collection or immediately thereafter, in order to prevent sample misidentification and ensure traceability throughout the diagnostic process.

Evidence compilation update:

Current guidelines build upon established protocols while introducing nuanced recommendations aimed at improving preanalytical quality. The SHEA-endorsed, medium-quality guideline (Suleyman et al., 2024) identifies three critical factors in reducing specimen rejection: accurate labelling, specimen stability, and compliance with transport requirements.

Complementing this, the low-quality guideline from the Clinical Excellence Commission (Clinical Excellence Commission, 2024a) provides practical guidance by explicitly warning against label

placement that obscures barcodes or the base of culture bottles, a frequent but often overlooked preanalytical error that can hinder automated processing and compromise traceability.

Key Recommendations Summary
Essential Information Required

- Mandatory data elements that must be recorded at time of collection:
  - Collection site (venipuncture location)
  - Date and exact time of collection
  - Requisitioning clinician/unit contact details

Quality Assurance Protocols

1. Real-time Documentation
   a. Labels must be applied immediately post-collection to prevent specimen mix-ups
2. Label Placement Standards
   a. Never obscure:
      i. Manufacturer barcodes
      ii. Bottle base visibility (for volume inspection)
3. Rejection Prevention
   a. Three critical compliance factors:
      i. Accurate labeling
      ii. Specimen stability maintenance
      iii. Proper transport condition

4.3.3.4 Conservation of the blood culture bottles until transportation

Evidence in reference document:

In the reference INESSS guide, all the documents included recommended keeping blood samples at room temperature at all times. Some of them specified that the samples should not be refrigerated, freeze or stored at temperatures above 30º C (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; Gorski et al., 2021; De Plato et al., 2019; OPTMQ, 2018).

Evidence compilation update:

6 documents found discussed the conservation of bottles until laboratory transportation. Out of this guidelines, two of medium-quality (Fontana et al., 2023; Suleyman et al., 2024) and the remain of low-quality (Clinical Excellence Commission, 2024a, 2024c; NHS England, 2023; Rodríguez Díaz et al., 2017) report the same evidence as the INESSS reference guide, as they specified that bottles should be kept at room temperature at all times. 3 of these guidelines (Fontana et al., 2023; NHS England, 2023; Rodríguez Díaz et al., 2017) specified that blood culture bottles should be placed in the incubator within 2 hours of the extraction.

Key Recommendations Summary

Temperature Requirements

- Maintain at room temperature (15–30°C) at all times
- Never:
  - Refrigerate
  - Freeze
  - Expose to temperatures >30°C

Time to Processing

- Transport to lab immediately after collection
- If delay occurs:
  - Max 2 hours at room temperature before incubation
  - Prioritize placement in incubator within this window

4.3.3.5 Other sampling consideration

Evidence compilation update:

Current literature across evidence levels supports blood diversion strategies as effective interventions. While high/medium-quality studies (Bonomo et al., 2024; Suleyman et al., 2024) focus on the 1 mL diversion protocol's efficacy in reducing false positives, a low-quality review (Callado et al., 2023) expands the implications to broader healthcare outcomes, from antibiotic stewardship and length of stay reduction to mortality and cost benefits stemming from fewer contaminated specimens.

Key Recommendations Summary
Diversion protocols enhance diagnostic accuracy and may improve clinical outcomes.

4.4 Post-extraction

4.4.1 Rapid tests

Evidence in reference document:

The INESSS reference guide did not explore the rapid tests topic.

Evidence compilation update:

A high-quality (Bonomo et al., 2024) and a medium-quality guideline (Fontana et al., 2023) highlight the increasing integration of rapid molecular assays (RMAs) performed **after a blood culture turns positive**. These tests enable identification of a predefined set of pathogens within approximately two hours, assisting clinicians in distinguishing true infections from contaminants (e.g., skin flora). Additionally, molecular assays can detect antibiotic resistance-conferring genes (e.g. carbapenemases, extended-spectrum beta-lactamases, methicillin resistance) thereby supporting faster and more accurate clinical decision-making. In this context, RMAs are considered valuable adjuncts to conventional blood culture workflows, enhancing diagnostic timeliness without replacing standard methods.

A key **rapid diagnostic approach performed on positive blood cultures involves:**

- Rapid identification of the pathogen through differential centrifugation followed by MALDI-TOF mass spectrometry analysis of the cell pellet. This method yields results within approximately one hour after Gram stain, and is applicable primarily to monomicrobial blood cultures.
- Rapid antimicrobial susceptibility testing (RAST) using the EUCAST methodology, which provides preliminary zone diameter data after 4, 6, 8, and 16 hours of incubation directly from the positive blood culture bottle.

This is currently among the most cost-effective and widely implemented rapid diagnostic strategies in clinical microbiology laboratories. However, as with any rapid diagnostic tool, its clinical impact depends critically on two conditions:

1. The Microbiology laboratory must operate 24/7, and
2. Results must be communicated rapidly and directly to the treating clinician, such as those in the Emergency Department, Internal Medicine, or Intensive Care Unit, so that timely therapeutic decisions can be made.

On the other hand, a high-quality systematic review (Rapszky Anna et al., 2025) examines RMAs performed **directly from whole blood**, evaluating their potential to replace blood cultures. While these direct-from-blood RMAs show promise, particularly in patients already started on antimicrobial therapy (AMT), the review concludes that their sensitivity and specificity are currently insufficient to replace traditional blood cultures. While these assays may have a supplementary role in selected clinical scenarios, such as immunocompromised patients or suspicion of infection by fastidious or non-culturable pathogens, further validation is necessary before they can be recommended for routine use.

The review also identifies key limitations of direct molecular testing, such as higher cost, technical complexity, and the requirement for large blood volumes, which may not be feasible or beneficial across all patient populations. Current evidence does not support updating clinical guidelines to prioritize RMAs over conventional blood cultures.

A high-quality narrative review (Samuel, 2023) evaluates the diagnostic and clinical utility of advanced molecular techniques, namely nucleic acid amplification tests (NAAT) and next-generation sequencing (NGS), applied directly from whole blood. These technologies aim to overcome the limitations of conventional blood cultures by enabling faster detection of a broader range of pathogens, including non-culturable or fastidious organisms. The review emphasizes that NAAT platforms, such as FAST-ID and SepsiSTAT, offer promising turnaround times (as fast as 30 minutes to 4 hours), while NGS provides comprehensive microbial and resistance profiling, albeit with longer processing times and greater technical demands. Despite their potential, the review concludes that neither NAAT nor NGS currently demonstrate sufficient sensitivity, specificity, or impact on patient outcomes to warrant routine use as standalone diagnostic tools. Instead, they are best considered as adjunctive methods, particularly in complex or high-risk cases (e.g., culture-negative sepsis or immunocompromised patients). Additional concerns include the potential for false positives due to detection of non-viable DNA, the absence of standardized interpretation criteria, and the high costs associated with NGS, often requiring significant reductions in hospital length of stay to achieve cost-effectiveness.

In summary, while RMAs offer valuable rapid diagnostic capabilities, their role depends on the clinical context and results should always be assessed along with other microbiological tests. RMAs performed after blood culture positivity can significantly enhance timely identification of pathogens and resistance mechanisms, while direct-from-blood molecular tests remain largely investigational and should be considered complementary until further evidence supports broader implementation. For now, blood cultures continue to be the gold standard for diagnosing bloodstream infections.

Key Recommendations Summary

Current Role of RMAs

- Valuable adjunct to traditional blood cultures, providing rapid pathogen identification (<2 hours).
- Not a replacement for blood cultures due to insufficient sensitivity.
- Limitations: Direct-from-blood RMAs show promise but lack sufficient sensitivity and specificity to replace blood cultures in routine practice.

Optimal Use Cases

- **After Blood Culture Positivity**:
  - Pathogen identification via MALDI-TOF mass spectrometry (~1 hour post-Gram stain).
  - Rapid antimicrobial susceptibility testing (RAST) using EUCAST guidelines for preliminary resistance data (within 4–16 hours).
- **Direct-from-Blood Molecular Assays**:
  - Potential supplementary role for:
    - Fastidious/non-culturable pathogens.
    - Patients already on antimicrobial therapy (AMT).

- High-risk populations (e.g., immunocompromised patients).

Limitations & Challenges

- Higher costs and larger blood volume requirements
- No current evidence to support replacing standard blood culture protocols

Future Directions

- Research needed to improve:
  - Pathogen coverage
  - Sensitivity
  - Sample volume optimization

4.4.2 Microbiology laboratory considerations

Evidence in reference document:

No further considerations were discussed in the INESSS reference guide.

Evidence compilation update:

A low-quality guideline (Rodríguez Díaz et al., 2017) outlines several key steps for handling blood cultures upon arrival at the laboratory. First, it emphasizes the critical need to verify patient and sample identification to prevent errors. Once registered, bottles should be immediately placed in incubators to ensure optimal conditions for microbial growth. The guideline also highlights the importance of IT systems that enable automatic, bidirectional communication between the microbiology lab and treating physicians, facilitating faster clinical decision-making. Additionally, it suggests reviewing previous or concurrent cultures, even from other facilities, as this may help determine the source of bacteraemia/fungemia and aid in microorganism identification.

In a more recent medium-quality guideline (Suleyman et al., 2024), further recommendations are provided to enhance blood culture reliability. Laboratories should implement a quality control system that monitors blood culture volumes and provides feedback to collectors to ensure proper technique. This system should track key metrics, including contamination rates and bottle fill volumes, as these factors significantly impact diagnostic accuracy. By maintaining rigorous oversight, labs can reduce false positives and improve the overall reliability of blood culture results.

Key Recommendations Summary

Specimen Handling Protocol

- Immediate verification of patient/sample identification
- Prompt incubation after registration (no delays)
- IT integration for real-time lab-clinician communication
- Cross-check with historical cultures (including external records)

Quality Control Measures

- Implement monitoring systems for:
  - Blood culture volumes (optimal fill compliance)
  - Contamination rates
- Provide feedback loops to collectors for technique improvement

Operational Priorities

1. Pre-analytical phase
   a. Ensure proper collection volumes (avoid under/overfilling)
   b. Minimize pre-processing delays
2. Analytical phase
   a. Standardize incubation protocols
   b. Utilize automated alerts for positive cultures
3. Post-analytical phase
   a. Correlate results with clinical context
   b. Flag potential contaminants based on clinical data

4.5 Follow up

4.5.1 Interpretation of the results

Evidence in reference document:

In the reference INESSS guide, some different situations were considered:

Risk of false negatives

The literature assessed by the INESSS reference document consistently identifies three primary factors contributing to false-negative blood culture results: pre-analytical collection conditions, insufficient sample volume, and the specific causative microorganism (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; De Plato et al., 2019; Doern et al., 2019; OPTMQ, 2018). Current evidence demonstrates remarkable consensus across multiple guidelines regarding these key determinants of culture sensitivity.

Risk of false positives

The distinction between a true positive blood culture result and a false positive or contamination is discussed in three papers (CLSI, 2022; De Plato et al., 2019; Doern et al., 2019). These sources collectively acknowledge the absence of definitive diagnostic criteria for this distinction. However, certain information relating to blood culture bottles, sampling, and the clinical picture may help to make a difference. Various clinical situations at risk of false positive results are presented.

Bacterial species

Four key guidelines address the identification of bacterial species in blood cultures (Miller et al., 2024; UKHSA, 2023; CLSI, 2022; Doern et al., 2019). While these documents show consensus regarding typical contaminant organisms, the UKHSA (2023) guideline provides particularly detailed characterization of pathogenic species.

Notably, two sources (UKHSA, 2023; Doern et al., 2019) emphasize an important clinical nuance: organisms conventionally considered contaminants may represent true bacteremia in specific patient populations, particularly in immunocompromised hosts and cases of suspected infective endocarditis. Gram stain results are not discussed in the selected papers.

Evidence compilation update:

2 documents found discussed this topic.

The interpretation of positive blood cultures remains a significant pain point in clinical practice. A recent survey (Duguid et al., 2025) paints a concerning picture: only 7% of physicians report feeling consistently confident when faced with preliminary positive blood culture results. This striking statistic reveals a critical gap in our diagnostic processes, particularly alarming given how heavily antibiotic decisions rely on these results.

The laboratory workflow for positive blood cultures should prioritize rapid processing. According to SEIMC (Rodríguez Díaz et al., 2017) in a low-quality evidence document, immediate Gram staining and subculture on appropriate media are essential first steps. For monomicrobial infections, direct identification and antimicrobial susceptibility testing should be performed to minimize turnaround time.

Effective interpretation requires integration of microbiological data with clinical context. The guideline emphasizes the importance of considering:

- Patient history and underlying conditions
- Current antimicrobial therapy
- Immune status and risk factors for infection

The guideline also provides specific recommendations for potential contaminants:

1. Microorganisms typically considered commensals should not be automatically dismissed

2. Interpretation should evaluate:
    a. Number of positive cultures
    b. Clinical correlation
    c. Patient-specific risk factors

A medium-quality guideline (Fontana et al., 2023) specified that close communication between microbiologists and clinicians is critical throughout this process to ensure appropriate clinical correlation and timely therapeutic decisions, and that this communication should be traceable. It is also essential to accurately record the date and time of incubation start and the date and time of positivity for each bottle (time to detection [TTD]). The report, generally electronic, must contain clear information on the entire diagnostic process performed in the laboratory, and must be easy to read and well-organized so that various elements (time to positivity, Gram stain, identification of the etiological agent, any associated resistance genes and/or mechanisms, and antimicrobial susceptibility testing results) are easily interpretable by clinicians. Considering the importance of microbiological data in managing patients with bloodstream infections, the generation of one or more preliminary reports before the final report is recommended.

Paediatric:

A low-quality guideline (Rodríguez Díaz et al., 2017) highlights the diagnostic challenge in differentiating true bacteraemia from contamination, particularly in paediatric patients when only a single blood culture sample is usually obtained. This limitation underscores the critical importance of strict aseptic technique during sample collection to minimize contamination risk. The guideline recommends obtaining an additional sample when clinical uncertainty exists regarding the significance of isolated microorganisms.

Key Recommendations Summary

False-Negative Risks

- Main causes:
    - Improper collection (pre-analytical errors)
    - Insufficient blood volume
    - Fastidious microorganisms

False-Positive/Contamination Risks

- Suggestive of contamination if:
    - Only 1/4 bottles positive
    - Skin flora organism (e.g., coagulase-negative staphylococci)
    - No foreign body/implant
- True bacteremia possible in:
    - Immunocompromised patients

- Suspected endocarditis

Interpretation Approach

1. Correlate with clinical context:
    a. Patient history, immune status, antimicrobial therapy
2. Lab processing:
    a. Immediate Gram stain & subculture
    b. Direct ID/AST for monomicrobial infections
3. Consult experts if uncertain (ID/microbiologist)

Pediatric Considerations

- Single cultures increase interpretation difficulty → prioritize aseptic technique.
- Repeat sampling if clinical doubt exists.

4.5.2 Follow-up blood cultures

Evidence in reference document:

In the reference INESSS guide, two documents discussed the general terms of the follow-up blood cultures (CLSI, 2022; Fabre et al., 2020b). It was stated that in general cases, follow-up blood cultures are not required. The follow-up blood cultures may be useful in clarifying the results of initial blood cultures in symptomatic individuals and to report on clinical situations where follow-up blood cultures should be taken to document eradication of the bacteraemia. In addition, one document recommends waiting two to five days before taking follow-up blood cultures to allow time for the antibiotic to take effect (CLSI, 2022).

Evidence compilation update:

As in the reference INESSS guide, a medium-quality guideline (Fontana et al., 2023) specified that there are no indications for follow-up blood cultures, as follow-up should be based on clinical data. It was added that blood cultures should not be repeated before three days from the start of targeted therapy with three exceptions: (1) endocarditis; (2) Staphylococcus aureus sepsis, where repeat cultures after 2 and 4 days can provide useful information on infectious complications arising via hematogenous spread; (3) candidemia.

According to a low-quality document (Rodríguez Díaz et al., 2017), repeat blood cultures should be considered in other two key scenarios: (1) when the infection focus remains unclear after initial negative cultures, with repeat sampling recommended between 48-72 hours; and (2) to assess treatment response in confirmed bacteraemia, with repeat cultures at 48-72 hours to evaluate microbiological persistence.

For emergency department settings, a high-quality guideline (Schoffelen et al., 2024) emphasizes the importance of implementing structured follow-up protocols for blood culture results after patient discharge, ensuring appropriate clinical review and management.

In specific patient populations, the evidence varies:

- For neutropenic patients, a medium-quality guideline (Suleyman et al., 2024) recommends against routine daily blood cultures in clinically stable patients with persistent fever after initial evaluation.
- A low-quality guideline (Clinical Excellence Commission, 2024b) provides detailed indications for repeat blood cultures, strongly recommending them for:
    - Staphylococcus aureus or Candida bloodstream infections (to confirm clearance)
    - Persistent sepsis >48 hours on appropriate antibiotics
    - Single positive cultures with skin contaminants in patients with intravascular devices/prostheses
    - Suspected endocarditis or high-risk endovascular infections

The same guideline (Clinical Excellence Commission, 2024b) notes situations where repeat cultures are not routinely indicated:

- Known infections without source control (unless new sepsis symptoms develop)
- Uncomplicated Gram-negative bacteraemia from a known source in stable patients
- Febrile neutropenia without new infectious symptoms

These recommendations highlight the importance of clinical judgment and, when uncertain, consultation with infectious disease or microbiology specialists. The variation in evidence quality (from low to high) underscores the need for further research to refine these clinical guidelines, particularly for specific patient populations and clinical scenarios.

Paediatric:

A low-quality document considered that serial blood culture collection is not recommended, except in immunosuppressed paediatric patients (Rodríguez Díaz et al., 2017).

Key Recommendations Summary

General Approach

- Not routinely needed in most cases.
- Consider if:
    - Unclear initial results + ongoing symptoms
    - Documenting bacteraemia clearance (e.g., S. aureus, Candida)
- Timing:
    - First follow-up: 48–72h after antibiotics (or after initial results if concerns)

- Avoid repeats before 3 days targeted therapy, except for:
  - Endocarditis
  - S. aureus sepsis (repeat at 2 & 4 days to monitor complications)
  - Candidemia

High-Risk Scenarios Requiring Follow-up

- Persistent sepsis (>48h on appropriate antibiotics)
- S. aureus, Candida, or endovascular infections (e.g., endocarditis)
- Paediatric:
  - <2 months old with bacteraemia
  - Immunosuppressed children (only exception for serial cultures)

When to Avoid

- Uncomplicated Gram-negative bacteraemia (known source, stable patient)
- Febrile neutropenia without new symptoms
- Known infection with unresolved source control (unless sepsis develops)

Clinical Judgment

- Always prioritize patient context over protocolized testing.

4.5.3 Contamination considerations

Evidence in reference document:

No further considerations were discussed in the INESSS reference guide regarding the contamination of blood cultures.

Evidence compilation update:

Multiple aspects of blood culture contamination prevention were addressed in the literature, particularly:

Interpreting Potential Contaminants

The medium-quality guideline (Fontana et al., 2023) provides crucial laboratory guidance for handling suspected contaminants. Nevertheless, it is usually difficult to definitively classify a contaminant, and the significance of isolating these microorganisms must always be interpreted within the clinical context. Even when contamination is suspected, microorganism identification must be performed.

- Microorganisms isolated from only one bottle or one set out of multiple collections should typically be considered contaminants.

- Common contaminants include coagulase-negative staphylococci, *Corynebacterium spp*., and *Cutibacterium* acnes.

Tracking and Monitoring Contamination Rates:

- A survey (Duguid et al., 2025) recommended recording contamination rates for quality improvement, linking it to cost savings.
- A low-quality document (Bunn & Cornish, 2025) emphasized monitoring as a proxy for protocol effectiveness, particularly in elderly patients, that have higher contamination risk.

Proper Venipuncture Preparation and Dedicated Phlebotomy Teams:

- A medium-quality guideline (Suleyman et al., 2024) stressed antisepsis and specialized phlebotomy teams to reduce skin flora contamination.
- A high-quality document (Bonomo et al., 2024) and a high-quality systematic review (Sautter et al., 2024) reinforced the benefits of dedicated phlebotomy teams.

Rapid Diagnostic Tests and Diversion Devices:

- High/medium-quality evidence (Bonomo et al., 2024; Suleyman et al., 2024)supported rapid tests to distinguish pathogens from contaminants.
- Several high-quality review (Sautter et al., 2024) endorsed diversion devices for peripheral blood cultures.

Staff Training and Feedback

- The medium-quality guideline (Suleyman et al., 2024) recommended monitoring with staff education.
- A low-quality guideline (Rodríguez Díaz et al., 2017) advised training and protocol adherence, noting peripheral venipuncture's lower contamination rates but debunking discarding the first mL of blood.

Special Considerations for Elderly Patients

- The low-quality document (Bunn & Cornish, 2025) highlighted elderly patients' higher contamination susceptibility.

Key Recommendations Summary

Contaminant Interpretation

- Suspect contamination if:
  - Isolated from only 1 bottle or 1/2–3 sets.
  - Typical contaminants: Coagulase-negative staphylococci, *Corynebacterium spp.* or *Cutibacterium acnes.*
- Required actions:
  - Always identify microorganisms (species-level).
  - Clinical correlation mandatory (never dismiss based solely on lab criteria).
  - Report with contaminant warning, even if susceptibility testing is later requested.

Monitoring

- Track contamination rates.

Collection Technique

- Use dedicated phlebotomy teams.
- Strict antisepsis protocols.

Technology

- Implement rapid diagnostic tests and initial specimen diversion devices.

Staff Training

- Regular staff education and performance feedback.

High-Risk Groups

- Enhanced protocols for elderly patients.

4.6 Environmental issues

Evidence in reference document:

While the INESSS reference guide did not provide evidence of environmental concerns, its expert panel addressed this debated issue, including discussions on electricity costs and ecological impact. Blood cultures contribute to healthcare's carbon footprint through waste generation (e.g., single-use plastics), electricity consumption (e.g., incubators), transportation (equipment, samples, and staff travel), report production, and laboratory operations.

Key Recommendations Summary

- *Ensuring appropriate prescription:* Ensuring blood cultures are ordered only in appropriate clinical contexts aligns with broader goals of diagnostic stewardship and environmentally sustainable healthcare.
- *Responsible use blood cultures:* Decoupling blood cultures from routine septic work-up requests could further reduce unnecessary testing.

4.7. Considerations for National and EU-Level Implementation

Two authoritative sources, the U.S. Centers for Disease Control and Prevention (CDC) in their report on blood culture contamination and diagnostic stewardship (Bunn & Cornish, 2025), and NHS England in their executive summary on improving the blood culture pathway (NHS England, 2023),

proposed key measures aimed at enhancing the accuracy, safety, and efficiency of blood culture practices. These documents offer evidence-informed considerations that can guide national or EU-level health policy development under the leadership of the European Commission and the European Center for Disease Prevention and Control (ECDC).

1. **Standardization of Blood Culture Collection Practices**

Both the CDC (2025) and NHS England (2023) identify variability in blood culture collection as a key contributor to contamination and diagnostic inaccuracy. The CDC points to inadequate skin antisepsis and incorrect blood volumes as common failings, while NHS England highlights inconsistent practices across care settings as a source of avoidable delays and inappropriate antimicrobial use.

Considerations for National and EU-level implementation:

Based on the evidence findings, it could be valuable to promote the adoption of standardized, evidence-based blood culture collection protocols across Member States to reduce contamination, improve diagnostic reliability, and strengthen antimicrobial stewardship efforts.

2. **Establishment of National Quality Indicators – Strengthening monitoring**

Both the CDC (2025) and NHS England (2023) emphasize the need to monitor blood culture quality through specific indicators. The CDC has formally adopted blood culture contamination rates, with a target threshold of ≤3%, as a national patient safety measure. NHS England recommends implementing local audits to track two key pre-analytical indicators: (1) collection-to-incubator time and (2) blood sample volume. These are to be regularly reviewed at organizational level, reported nationally, and potentially integrated into accreditation processes. NHS also highlights the importance of regional AMS leads, that could help organizations to develop and implement strategies that could be shared as standardized practice across networks or regions, reducing variation in practice.

Considerations for National and EU-level implementation:

Considering the evidence, it would be beneficial to support the development of harmonized quality indicators for blood culture performance, including contamination rates, sample volume, and time to incubation, and encourage routine monitoring across Member States. These indicators could serve as a foundation for continuous improvement, benchmarking, and integration into national patient safety and accreditation systems.

3. Mandatory Education and Competency-Based Training

Both the CDC (2025) and NHS England (2023) emphasize the essential role of continuous education and competency assessments for healthcare workers involved in blood culture collection. Ensuring staff are properly trained reduces contamination risks and supports adherence to standardized protocols.

Considerations for National and EU-level implementation:

The evidence suggests the importance of promoting the establishment of recurring training programmed and certification processes for frontline staff responsible for blood culture collection across Member States, with systems in place to monitor compliance and maintain competency standards.

4. Multidisciplinary Leadership and Governance

NHS England (NHS, 2023) stresses the importance of embedding antimicrobial stewardship and infection prevention priorities into clinical leadership and governance structures. This includes establishing multidisciplinary quality improvement teams involving microbiologists, sepsis leads, AMS/AMR experts, clinicians, and support staff. Improvement efforts should be integrated within existing governance frameworks, ensuring continuous quality improvement from "board to ward."

Considerations for National and EU-level implementation:

Based on the evidence findings, it could be valuable to encourage Member States to integrate blood culture quality improvement into existing governance frameworks for AMR, sepsis, and infection prevention, fostering multidisciplinary leadership and use of audit data to drive improvements.

Key Recommendations Summary

1. Standardize Collection Protocols

- Goal: Reduce contamination and improve diagnostic accuracy
- Actions:
    - Adopt evidence-based guidelines for:
        - Skin antisepsis
        - Optimal blood volumes
        - Sample handling
    - Harmonize practices across care settings to minimize variability

2. Implement Quality Indicators

- Core Metrics:

    - Contamination rates (target ≤3%)
    - Collection-to-incubation time
    - Sample volume adequacy
- Monitoring:
    - Integrate into national patient safety programs
    - Support local audits with regional/national benchmarking

3. Enhance Education & Training

- Mandate:
    - Competency-based training for all staff performing cultures
    - Recertification programs to maintain standards
- Support:
    - EU-wide training frameworks adaptable to local contexts

4. Strengthen Governance

- Multidisciplinary Leadership:
    - Involve microbiologists, AMS teams, sepsis leads, and clinicians
    - Embed blood culture quality into existing AMR/sepsis governance
- Accountability:
    - Link performance metrics to accreditation systems
    - Foster "board-to-ward" responsibility for improvement

EU-Level Opportunities:

- Align with One Health AMR strategies
- Leverage ECDC coordination for cross-border benchmarking

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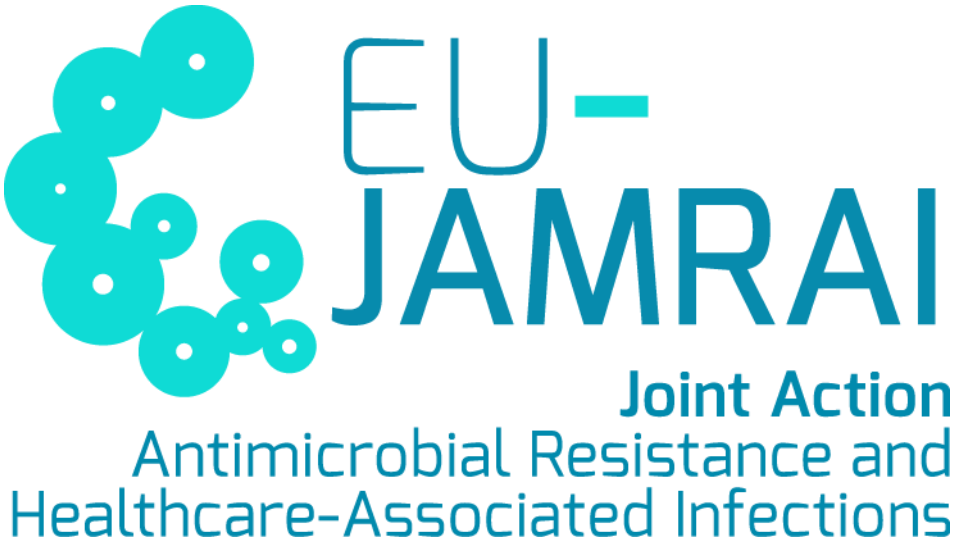

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