



ISSN 2545-2533

Received: 03.07.2026

Accepted: 10.09.2026

First online: 11.09.2026

Published: 30.09.2026

## Culture-negative sepsis: diagnostic approach and emerging molecular tests

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### ABSTRACT

Diabetic ketoacidosis (DKA) is usually precipitated by insulin deficiency, infection, or physiologic stress, but acute poisoning and drug exposure may precipitate, aggravate, or obscure DKA. Toxicologic conditions may also mimic or coexist with DKA. Culture-negative sepsis is a common and clinically difficult syndrome in which patients have sepsis or septic shock, but routine cultures do not recover a causative pathogen. Negative cultures should not be interpreted as proof that infection is absent, particularly after previous antimicrobial exposure, low-volume or delayed blood-culture collection, localized infection, or infection caused by fastidious, viral, fungal, mycobacterial, zoonotic, or intracellular organisms. At the same time, the culture-negative label can create diagnostic anchoring: noninfectious mimics such as pancreatitis, pulmonary embolism, adrenal crisis, malignancy-related inflammation, cytokine release syndrome, hemophagocytic lymphohistiocytosis, and drug reactions may present with fever, inflammatory markers, and organ dysfunction. This review offers a practical approach to culture-negative sepsis, emphasizing early resuscitation, optimized conventional microbiology, source-directed imaging and sampling, reassessment at 48-72 hours, antimicrobial stewardship, and selective use of molecular and host-response diagnostics. Rapid positive blood-culture panels, direct-from-blood targeted assays, microbial cell-free DNA metagenomic sequencing, and transcriptomic host-response tests may improve diagnostic yield or shorten time to actionable information. Their value depends on pretest probability, specimen quality, organism spectrum, resistance interpretation, turnaround time, cost, and integration with infectious diseases and antimicrobial stewardship teams. Molecular results should be treated as complementary evidence, not as substitutes for cultures, source control, or bedside clinical judgment.

**KEY WORDS:** Sepsis, septic shock, culture-negative sepsis, molecular diagnostics, metagenomic next-generation sequencing, antimicrobial stewardship.

## INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. It remains a major global health problem; the global burden of disease (GBD) analysis estimated 48.9 million incident cases and 11.0 million sepsis-related deaths worldwide in 2017 [2]. The first clinical priority is rapid recognition and treatment. The 2026 Surviving Sepsis Campaign (SSC) guidelines emphasize collecting blood cultures as soon as possible and ideally before antimicrobial therapy, immediate resuscitation, timely antimicrobials according to the likelihood and severity of infection, early source control, and ongoing reassessment with antimicrobial de-escalation when appropriate [3].

Early sepsis care also begins before hospital arrival. The 2026 SSC guidelines explicitly address the immediate prehospital setting and suggest use of a standardized sepsis screening tool for acutely ill adults transported by ambulance or flight [3]. Once sepsis is suspected, out-of-hospital (OOH) priorities include supportive oxygenation when hypoxemia is present, rapid vascular access, early crystalloid resuscitation for sepsis-induced hypoperfusion, prompt transport, and pre-arrival sepsis notification to the receiving emergency department. For patients with probable or definite sepsis and hypotension, prehospital antimicrobials may be considered when the anticipated time to in-hospital evaluation exceeds 60 minutes and a structured screening process is in place [3]. Screening tools such as the Modified Early Warning Score (MEWS) and quick Sequential Organ Failure Assessment (qSOFA) have shown variable performance in the prehospital setting and should support, rather than replace, clinical assessment [4]. End-tidal carbon dioxide (EtCO<sub>2</sub>), when available, may provide additional noninvasive risk information, but it should likewise be interpreted as an adjunct rather than a stand-alone diagnostic test [5].

Culture-negative sepsis describes patients with a clinical syndrome of sepsis or septic shock in whom routine cultures fail to identify a pathogen. The term is useful at the bedside but biologically heterogeneous. It includes true infection missed by conventional tests, infection partially sterilized by earlier antibiotics, localized infection without bloodstream invasion, fastidious or non-cultivable pathogens, viral or fungal syndromes, inadequate sampling, and noninfectious mimics. Table 1 summarizes common reasons for negative cultures and the diagnostic implications.

The clinical importance of culture-negative sepsis is twofold. First, it is frequent. Reviews and cohort studies report that a substantial proportion of sepsis episodes have no microbiologic confirmation, with estimates varying according to setting, culture practice, prior antibiotic exposure, sepsis definition, and timing of sampling [6-10]. Second, prognosis is variable. Some cohorts report lower mortality than culture-positive sepsis, whereas others report similar or higher mortality, particularly among patients with septic shock or delayed appropriate therapy [7-11]. Therefore, negative cultures should neither reassure clinicians too early nor justify indefinite broad-spectrum therapy without reassessment. They should prompt a structured diagnostic and stewardship review.

**Table 1.** Why cultures may remain negative in patients with suspected sepsis.

| Category                                                     | Typical clues                                                                                                                                    | Diagnostic implications                                                                                                                                                                                                                                                                       |
|--------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Prior antimicrobial exposure                                 | Antibiotics started before cultures; outpatient antibiotics; recent hospitalization.                                                             | Cultures may be partially sterilized. Obtain cultures as early as possible, review prior antimicrobials, and consider molecular tests if the result would alter therapy.                                                                                                                      |
| Suboptimal culture collection                                | Low blood volume, only one set, cultures drawn after antibiotics, or line-only cultures without peripheral cultures.                             | Optimize conventional microbiology first: at least two properly filled sets, separate venipuncture sites, and paired peripheral and catheter cultures when catheter-related infection is possible.                                                                                            |
| Localized infection without bacteremia                       | Pneumonia, cholangitis, abscess, urinary obstruction, osteomyelitis, device infection, or necrotizing infection.                                 | Blood cultures can be negative. Use source-directed imaging and obtain high-quality site specimens before or during source control.                                                                                                                                                           |
| Fastidious or slow-growing bacteria                          | Culture-negative endocarditis, animal exposure, homelessness, valvular disease, prosthetic valve, or chronic symptoms.                           | Consider <i>Coxiella burnetii</i> , <i>Bartonella</i> spp., <i>Brucella</i> spp., <i>Tropheryma whippelii</i> , HACEK organisms, mycoplasmas, and anaerobes; use serology, PCR, tissue testing, and prolonged incubation when indicated.                                                      |
| Viral sepsis or severe viral infection                       | Respiratory outbreak, lymphopenia, hepatitis, myocarditis, encephalitis, or immunocompromised host.                                              | Use multiplex respiratory PCR, blood or site-specific viral PCR, and syndrome-directed testing; antibacterial de-escalation may be possible if bacterial infection becomes unlikely.                                                                                                          |
| Fungal or mold infection                                     | Neutropenia, transplant, total parenteral nutrition, central venous catheter, abdominal surgery, or persistent fever despite antibiotics.        | Blood cultures may miss molds and some deep fungal infections. Consider beta-D-glucan, galactomannan, fungal cultures, tissue biopsy, and early source control.                                                                                                                               |
| Mycobacterial, rickettsial, parasitic, or zoonotic infection | Travel, animal exposure, tick exposure, fever with thrombocytopenia, hepatitis, rash, or chronic wasting.                                        | Routine blood cultures are often negative. Use exposure history, serology, PCR, acid-fast cultures, malaria smear or rapid testing, and public health notification when relevant.                                                                                                             |
| Noninfectious sepsis mimics                                  | No convincing source, sterile cultures, poor antibiotic response, disproportionate inflammation, thrombosis, endocrine crisis, or drug exposure. | Reconsider pancreatitis, pulmonary embolism, adrenal crisis, mesenteric ischemia, hemophagocytic lymphohistiocytosis (HLH), cytokine release syndrome, drug reaction with eosinophilia and systemic symptoms (DRESS), vasculitis, malignancy-related inflammation, and transfusion reactions. |

### Epidemiology and outcomes: why the data appear conflicting

Culture-negative sepsis is not a single disease entity. A nationwide U.S. analysis of severe sepsis hospitalizations found that 47.1% were culture-negative and reported higher hospital mortality in culture-negative than culture-positive cases [7]. In contrast, a multicenter ICU study by Phua and colleagues found lower hospital mortality among culture-negative than culture-positive severe sepsis [8]. A retrospective septic shock cohort reported broadly similar outcomes between culture-negative and culture-positive shock, while also showing that delay in appropriate antimicrobial therapy increased mortality in both groups [9]. A systematic review and meta-analysis confirmed substantial heterogeneity across studies, reflecting differences in definitions, culture methods, illness severity, and patient populations [10].

Recent prospective data add further nuance. A 2024 Korean Sepsis Alliance study of ICU patients meeting Sepsis-3 criteria found that 37% were culture-negative; among patients with septic shock, culture-negative status was associated with higher adjusted mortality [11]. This supports a practical bedside principle: culture-negative septic shock should be treated as high risk until the patient improves, the source is controlled, or a convincing noninfectious diagnosis is established. Conversely, in clinically improving patients with repeated negative cultures and no ongoing source, the same culture-negative label should support antimicrobial narrowing or discontinuation rather than prolonged broad-spectrum treatment.

## A DIAGNOSTIC FRAMEWORK FOR CULTURE-NEGATIVE SEPSIS

The diagnostic approach must be time-dependent. In the first hour, the priority is recognition, resuscitation, cultures when feasible, and empiric antimicrobials when sepsis is likely. After initial stabilization, the question changes from “Is this sepsis?” to “What source, pathogen, or mimic best explains the syndrome, and can therapy be safely narrowed?” Table 2 outlines a practical sequence.

**Table 2.** Practical time-dependent approach to culture-negative sepsis.

| Time point                                         | Priority actions                                                                                                                                                                                                                                                                            | How this changes management                                                                                                                            |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| 0-1 hour: recognition and stabilization            | Assess organ dysfunction, lactate, shock, oxygenation, mental status, and urine output. Obtain blood cultures before antibiotics when this does not cause substantial delay. Start empiric antimicrobials according to severity and likely source.                                          | Avoids missing early septic shock while preserving the chance of microbiologic diagnosis.                                                              |
| 1-6 hours: source-directed diagnostics             | Repeat focused history and examination; review devices, wounds, lines, surgery, immunosuppression, travel, animal exposure, and prior antibiotics. Send urine, respiratory, stool, CSF, ascitic, pleural, wound, or drainage samples when clinically indicated. Image the suspected source. | Moves the case from generic broad-spectrum coverage to source-specific therapy and source control.                                                     |
| 6-24 hours: source control and risk stratification | Drain abscesses, relieve obstruction, remove infected catheters, debride necrotic tissue, and involve surgery, interventional radiology, infectious diseases, or critical care early. Consider syndrome-specific PCR or serology.                                                           | Culture-negative sepsis often persists because the source has not been sampled or controlled. Early source control is both diagnostic and therapeutic. |
| 24-48 hours: early culture-negative checkpoint     | Review preliminary cultures, Gram stains, imaging, medication exposures, biomarkers, and clinical trajectory. Reassess whether the syndrome is infection, sterile inflammation, or mixed pathology.                                                                                         | Prevents diagnostic anchoring when cultures remain negative and the patient is not responding as expected.                                             |
| 48-72 hours: stewardship decision point            | If improving and no pathogen or uncontrolled source is found, narrow or stop unnecessary agents according to syndrome and risk. If worsening, broaden the diagnostic search with repeat imaging, tissue or sterile-site sampling, and selective molecular tests.                            | Balances the risk of undertreating occult infection against the harm of unnecessary broad-spectrum therapy.                                            |
| >72 hours: persistent unexplained illness          | Evaluate fastidious organisms, fungal or mycobacterial disease, endocarditis, HLH, malignancy, vasculitis, thromboembolism, drug fever, and cytokine-mediated syndromes. Consider metagenomic sequencing or biopsy when actionable.                                                         | Culture-negative status becomes a reason for deeper diagnosis, not simply prolonged empiric antibiotics.                                               |

**Step 1: optimize conventional microbiology before declaring sepsis culture-negative**

The most correctable reason for negative cultures is inadequate sampling. Blood-culture yield depends on the volume of blood collected, the number of sets, timing before antimicrobials, and sampling from appropriate sites. State-of-the-art reviews emphasize that, in adults, collecting multiple adequately filled blood-culture sets is more valuable than repeatedly collecting low-volume cultures [12]. A practical standard is at least two sets from separate venipuncture sites before antibiotics when feasible. If catheter-related bloodstream infection is possible, paired peripheral and catheter cultures should be obtained. Non-blood cultures should match the clinical syndrome because indiscriminate sampling increases false positives, colonization findings, and unnecessary antibiotics. A patient should not be labeled definitively culture-negative after one low-volume culture set, after antibiotics were already started, or before relevant source samples were obtained. Similarly, negative blood cultures do not exclude pneumonia, intra-abdominal abscess, cholangitis, obstructive pyelonephritis, necrotizing soft-tissue infection, osteomyelitis, infected vascular grafts, or device-associated infection. In these settings, source sampling and source control often provide more diagnostic value than repeated blood cultures.

**Step 2: reassess the source and the host**

Culture-negative sepsis is more likely in patients with prior antibiotics, deep-seated infection, immunosuppression, neutropenia, malignancy, indwelling lines, prosthetic material, recent surgery, and atypical exposures. The clinical evaluation should deliberately search for occult sources: infected vascular catheters, endocarditis, biliary obstruction, renal obstruction, intra-abdominal collections, sinusitis in neutropenia, invasive fungal disease, pressure ulcers, postoperative collections, and central nervous system infection. In immunocompromised patients, more than one process may be present; fever may reflect infection, treatment-related inflammation, or both.

Host factors should guide test selection. Persistent shock despite broad-spectrum antibiotics in a patient with hematologic malignancy may justify fungal biomarkers, chest CT, bronchoscopy, and metagenomic testing. By contrast, a clinically improving non-immunocompromised patient with negative cultures, no source, and an alternative diagnosis may benefit more from antimicrobial discontinuation than from broad molecular testing.

**Step 3: actively search for noninfectious mimics**

The most dangerous error in culture-negative sepsis is assuming that all inflammation is infection. Noninfectious mimics can produce fever, hypotension, lactate elevation, leukocytosis, thrombocytopenia, acute kidney injury, encephalopathy, and respiratory failure. Important examples include pancreatitis, pulmonary embolism, adrenal crisis, acute mesenteric ischemia, bowel perforation before cultures become positive, transfusion reactions, drug fever, drug reaction with eosinophilia and systemic symptoms (DRESS), tumor fever, hemophagocytic lymphohistiocytosis (HLH), macrophage activation syndrome, vasculitis, and cytokine release syndrome. The distinction is especially difficult in oncology, transplant, and rheumatology patients, where infection and inflammation often coexist.

Biomarkers can support but cannot replace clinical reasoning. Procalcitonin (PCT) is not sufficiently sensitive to rule out sepsis at presentation. A 2025 systematic review and meta-analysis of adult emergency department patients reported pooled sensitivity of 0.62 and specificity of 0.80 for sepsis diagnosis, based on low-certainty evidence [13]. PCT is therefore best used as part of serial reassessment and antimicrobial discontinuation decisions rather than as a stand-alone diagnostic gatekeeper. Transcriptomic host-response assays are promising adjuncts: validation studies of SeptiCyte RAPID have reported rapid turnaround and discrimination between sepsis and noninfectious systemic inflammation, but these assays measure host response rather than the causative pathogen and do not provide susceptibility data [14,15].

## EMERGING MOLECULAR TESTS

Molecular diagnostics can be grouped into four clinically relevant categories: rapid tests from positive blood cultures, direct-from-blood targeted pathogen assays, broad metagenomic sequencing, and host-response assays. Their strengths, limitations, and best-use scenarios are compared in Table 3. The central principle is diagnostic stewardship: testing should be ordered when the result is likely to change antimicrobial selection, source-control decisions, isolation or public health actions, or the decision to stop antibiotics.

### RAPID PANELS FROM POSITIVE BLOOD CULTURES

Rapid multiplex PCR panels from positive blood-culture bottles are among the most mature molecular tools. They do not solve persistently negative blood cultures, but they substantially shorten organism identification and selected resistance-gene reporting once a bottle is positive. In a multicenter evaluation, the BIOFIRE BCID2 panel showed high sensitivity and specificity for on-panel Gram-positive bacteria, Gram-negative bacteria, and yeast from positive blood-culture specimens, with high agreement for selected antimicrobial resistance genes [16]. A 2024 evaluation also demonstrated rapid pathogen and resistance-gene detection from positive cultures compared with conventional methods that may require 48-72 hours for identification and susceptibility results [17]. Earlier randomized evidence showed that rapid blood-culture identification produces the greatest clinical value when paired with antimicrobial stewardship intervention rather than reported passively [18]. For culture-negative sepsis, the lesson from positive-culture panels is indirect but important: speed alone is not enough. A rapid result changes care only if the institution has a pathway for immediate interpretation, antibiotic narrowing or escalation, and communication with the treating team.

### DIRECT-FROM-BLOOD PATHOGEN ASSAYS

Direct-from-blood assays attempt to detect pathogens without waiting for blood-culture positivity. This approach is attractive in patients who received antibiotics before sampling, have low-grade or intermittent bacteremia, or require immediate antimicrobial decisions. However, direct blood testing faces technical challenges, including low pathogen burden, high human DNA background, inhibitors, contamination, limited organism panels, and incomplete resistance prediction [19].

**Table 3.** Molecular and host-response tests relevant to culture-negative sepsis.

| Modality                                                         | Best clinical use                                                                                                                                      | Strengths                                                                                                                                               | Limitations                                                                                                                                                               |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Positive blood-culture multiplex PCR panels (e.g., BCID2, ePlex) | Blood culture bottle is positive and rapid organism or resistance identification is needed.                                                            | Fast organism and selected AMR-gene identification after bottle positivity; improves time to targeted therapy when linked to stewardship.               | Not useful if blood cultures remain negative; limited to panel targets; AMR genes do not replace phenotypic AST.                                                          |
| Direct-from-blood targeted NAAT/T2 magnetic resonance assays     | Septic shock or high-risk infection when prior antibiotics or delayed cultures may reduce yield and on-panel organisms are clinically plausible.       | Can provide same-day pathogen information without waiting for culture positivity; may detect selected organisms despite antibiotics.                    | Limited organism spectrum; no full susceptibility profile; positive DNA signal requires clinical interpretation; outcome benefit depends on stewardship workflow.         |
| Plasma microbial cell-free DNA mNGS                              | Persistent culture-negative sepsis, immunocompromised hosts, suspected fastidious organisms, polymicrobial infection, or prior antimicrobial exposure. | Broad, culture-independent detection of bacterial, fungal, viral, and parasitic DNA; useful when standard cultures are negative or difficult to obtain. | Cannot always distinguish infection from colonization, transient DNAemia, contamination, or nonviable organisms; limited resistance prediction; cost and turnaround vary. |
| Whole-blood or plasma-plus-blood-cell mNGS                       | Suspected intracellular pathogens, viral infection, or mixed bloodstream/tissue infection where cellular fraction may add information.                 | May improve yield compared with plasma alone in selected settings and may support severity assessment.                                                  | Requires local validation; background human DNA, interpretation thresholds, and false positives remain important barriers.                                                |
| 16S/18S/ITS PCR or sequencing from sterile-site tissue/fluid     | Culture-negative endocarditis, prosthetic infection, abscess, osteomyelitis, meningitis, or infection after antibiotics.                               | High value when performed on normally sterile tissue or fluid and linked to a compatible syndrome.                                                      | Requires invasive sampling; contamination can mislead; usually limited resistance information.                                                                            |
| Host-response transcriptomic assays                              | Distinguishing infection-related sepsis from sterile systemic inflammation when the diagnosis is uncertain.                                            | Rapid host-response signal; may support antibiotic stewardship and triage.                                                                              | Does not identify pathogen or susceptibility; performance varies by population, timing, immunosuppression, and inflammatory comorbidities.                                |
| Companion biomarkers (PCT, CRP, fungal biomarkers)               | Serial reassessment, antimicrobial discontinuation, or targeted evaluation for fungal disease.                                                         | Widely available and useful as trends in context.                                                                                                       | Insufficient as stand-alone rule-in/rule-out tests; affected by renal failure, surgery, trauma, immunosuppression, and noninfectious inflammation.                        |

The T2 Bacteria panel is one of the best-studied direct-from-blood targeted platforms for selected bacterial pathogens. A diagnostic accuracy study reported rapid detection of major bloodstream pathogens

directly from whole blood [20]. A 2024 clinical study found a shorter time to pathogen detection with T2Bacteria than with blood culture and a shorter time to targeted therapy [21]. However, direct-from-blood testing should not be expected to reduce antibiotic exposure automatically. Its effect depends on pretest probability, panel coverage, clinician confidence, and stewardship response; a negative result is most useful when the tested organisms plausibly explain the syndrome and when clinicians have a defined de-escalation pathway.

## **METAGENOMIC NEXT-GENERATION SEQUENCING AND MICROBIAL CELL-FREE DNA**

Metagenomic next-generation sequencing offers broader pathogen detection than targeted panels. Plasma microbial cell-free DNA sequencing can detect bacterial, fungal, viral, and parasitic DNA without requiring viable organisms. This is particularly appealing in culture-negative sepsis after antibiotics, suspected fastidious infection, polymicrobial infection, immunocompromised hosts, and cases in which invasive sampling is difficult. Recent studies have reported improved pathogen detection with clinical metagenomic sequencing in sepsis and septic shock compared with standard culture-based approaches [22,23]. A 2025 multicenter study in 859 adults reported that infected-site mNGS increased pathogen detection compared with conventional microbiological testing, improved etiologic diagnosis and tailored therapy, and was associated with better early outcomes, although longer-term survival benefit was not clearly established [24].

The enthusiasm for mNGS should be balanced with its interpretation challenges. Detection of microbial DNA does not prove viable infection, particularly when organisms are low abundance, common contaminants, or inconsistent with the clinical syndrome. Conversely, a negative mNGS result does not exclude infection if the pathogen burden is low, the specimen is from the wrong compartment, the organism is under-represented in the database, or antimicrobial therapy has already reduced nucleic acid levels. Molecular results also rarely provide complete phenotypic susceptibility, so cultures and sterile-site sampling remain essential when feasible.

## **HOST-RESPONSE TESTING**

Host-response assays address a different question: is the inflammatory syndrome more consistent with infection-related sepsis or sterile inflammation? This distinction is central in culture-negative sepsis because many mimics do not require antibiotics. SeptiCytte RAPID and related transcriptomic tests use host gene-expression signatures rather than pathogen detection. Validation studies suggest that such tests may help discriminate sepsis from noninfectious systemic inflammation with rapid turnaround [14,15]. Their main limitation is that they do not identify a causative organism, antimicrobial resistance, or infection source. They are best viewed as adjuncts for diagnostic uncertainty and stewardship rather than stand-alone tests.

## **PRACTICAL INDICATIONS FOR MOLECULAR TESTING**

Molecular tests should not be ordered automatically for every culture-negative fever or every patient with suspected sepsis. A high-value test usually meets three conditions: the pretest probability of infection

remains meaningful, conventional tests are negative or delayed despite appropriate sampling, and the result will change management. Examples include septic shock after prior antibiotics, immunocompromised patients with persistent fever and organ dysfunction, suspected culture-negative endocarditis, severe pneumonia with negative routine tests, postoperative or intra-abdominal sepsis without a clear organism, suspected zoonotic or intracellular infection, and cases in which stopping broad-spectrum antibiotics is risky without additional evidence.

Specimen choice is crucial. Plasma mcfDNA may be useful for bloodstream or disseminated infection, but tissue, valve, abscess, cerebrospinal fluid, bronchoalveolar lavage, pleural fluid, synovial fluid, or bone biopsy may be superior when infection is localized. A molecular test from the wrong compartment can be falsely negative or misleadingly positive. Molecular testing should therefore complement, not replace, source-directed sampling and source control.

### ANTIMICROBIAL STEWARDSHIP IN CULTURE-NEGATIVE SEPSIS

Antimicrobial stewardship is not separate from sepsis care; it is part of safe sepsis care. Continuing broad-spectrum therapy indefinitely because cultures are negative is not safer. It increases the risk of drug toxicity, *Clostridioides difficile* infection, fungal superinfection, selection of resistant organisms, and diagnostic complacency. Unnecessary antimicrobial exposure also contributes directly to secondary infections and to the broader public health problem of antimicrobial resistance (AMR). Conversely, stopping antibiotics too early in persistent shock may be dangerous. The correct approach is a structured reassessment that accounts for severity, trajectory, immune status, source probability, and the quality of the initial diagnostic work-up.

A practical 48-72-hour stewardship checkpoint should include five questions: (1) Is the patient improving? (2) Was an adequate microbiologic work-up obtained before or soon after antimicrobials? (3) Is there a controlled or uncontrolled source? (4) Is there a more likely noninfectious diagnosis? (5) Would an additional test change therapy? If the patient is improving, cultures and imaging are negative, source control is not required, and a noninfectious diagnosis is plausible, narrowing or discontinuation should be considered. If the patient is deteriorating, the response should not be simply broader antibiotics; it should be renewed source search, source control, and selective advanced diagnostics.

### PROPOSED DIAGNOSTIC ALGORITHM

A practical algorithm begins with syndrome confirmation: suspected infection plus acute organ dysfunction should trigger immediate sepsis management. Before antibiotics, obtain adequate blood cultures and source samples if this does not delay therapy.

After empiric treatment, classify the patient by trajectory: improving, stable but unexplained, or worsening. Improving patients undergo narrowing, duration planning, and reconsideration of noninfectious diagnoses. Stable but unexplained patients undergo targeted testing based on syndrome and exposures.

Worsening patients require source-control reassessment, repeat imaging, invasive sampling when feasible, and molecular diagnostics if the result could change antimicrobial or procedural management. At every step, culture-negative status should be interpreted together with severity, immune status, prior antibiotics, specimen quality, and source probability.

## IMPLEMENTATION CONSIDERATIONS

Hospitals adopting molecular diagnostics for culture-negative sepsis should avoid a technology-first approach. The highest-yield programs define who can order testing, which syndromes are appropriate, which samples are acceptable, how results are reported, and how stewardship teams respond. Reports should distinguish organisms with strong clinical plausibility from likely contaminants or low-level signals of uncertain significance. The laboratory, infectious diseases service, ICU, emergency department, and antimicrobial stewardship program should share a clear protocol for escalation, de-escalation, repeat sampling, and documentation. Without this infrastructure, molecular testing may increase cost and confusion without improving antibiotic use or outcomes.

## LIMITATIONS OF CURRENT EVIDENCE

The evidence base for culture-negative sepsis is limited by inconsistent definitions, variable culture practices, differences in antibiotic exposure before sampling, and heterogeneous patient populations. Molecular diagnostic studies often use composite reference standards because routine cultures are imperfect, but composite standards can overestimate or underestimate true accuracy. Many studies measure diagnostic yield or time to result rather than patient-centered outcomes such as mortality, organ-support duration, antibiotic-free days, adverse drug events, or cost-effectiveness. Future trials should evaluate test-guided stewardship pathways rather than diagnostic platforms alone.

## CONCLUSIONS

Culture-negative sepsis is common, heterogeneous, and clinically important. Initial management should prioritize rapid recognition, resuscitation, optimized cultures, empiric antimicrobials, and source control. When cultures remain negative, clinicians should avoid both extremes: assuming that infection has been excluded or continuing broad-spectrum therapy without reassessment. A structured approach should verify sampling quality, search for occult sources, consider unusual pathogens, identify noninfectious mimics, and use molecular diagnostics selectively. Rapid positive blood-culture panels, direct-from-blood assays, mNGS, microbial cell-free DNA sequencing, and host-response tests are useful additions to the diagnostic toolbox, but they are most valuable when embedded in diagnostic and antimicrobial stewardship. The future of culture-negative sepsis care is not one universal test; it is a disciplined pathway that combines bedside probability, source-directed sampling, molecular evidence, and timely therapeutic decisions.

## MESSAGE

- Culture-negative sepsis is common and should be managed initially as true sepsis when suspected infection and acute organ dysfunction are present.
- A negative culture is a diagnostic checkpoint, not a final diagnosis. It should trigger reassessment of sampling quality, prior antibiotics, source probability, unusual pathogens, and noninfectious mimics.
- Blood cultures remain essential because they provide viable organisms for phenotypic susceptibility testing; molecular tests can add speed or yield but cannot fully replace cultures.
- Molecular diagnostics are most useful when targeted to high-risk scenarios, such as prior antimicrobial exposure, immunocompromise, persistent shock, suspected fastidious infection, endocarditis, deep-seated infection, and decisions with major antimicrobial consequences.
- Diagnostic stewardship is as important as test selection: results require clinical adjudication, source-control planning, and a pathway for escalation, narrowing, or discontinuation of antimicrobials.

## ABBREVIATIONS

AMR, antimicrobial resistance; AST, antimicrobial susceptibility testing; BCID, blood-culture identification; CRP, C-reactive protein; DRESS, drug reaction with eosinophilia and systemic symptoms; ED, emergency department; EMS, emergency medical services; EtCO<sub>2</sub>, end-tidal carbon dioxide; HLH, hemophagocytic lymphohistiocytosis; ICU, intensive care unit; MEWS, Modified Early Warning Score; mcfDNA, microbial cell-free DNA; mNGS, metagenomic next-generation sequencing; NAAT, nucleic acid amplification test; OOH, out-of-hospital; PCT, procalcitonin; PCR, polymerase chain reaction; qSOFA, quick Sequential Organ Failure Assessment; SOFA, Sequential Organ Failure Assessment; SSC, Surviving Sepsis Campaign.

## SUPPLEMENTARY INFORMATION

**Funding:** No fund was received related to this study.

**Institutional Review Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data sharing is not applicable because no datasets were generated or analyzed for this narrative review.

**Conflicts of Interest:** The author declare no conflicts of interest.

**Ethics Approval:** Not applicable. This narrative review did not involve human participants, patient data, animal subjects, or experimental procedures.

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