Clinical Microbiology and Infection 31 (2025) 1993–2000

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.org

Position paper

The role of rapid and advanced microbiological methods in critical care: 2025 Emanuele Russo Delphi consensus

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https://doi.org/10.1016/j.cmi.2025.08.022

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ARTICLE INFO

Article history:
Received 17 April 2025
Received in revised form
12 August 2025
Accepted 17 August 2025
Available online 28 August 2025

Editor: L. Leibovici

Keywords:
Consensus
Critical care
Delphi
Microbiology
Rapid

ABSTRACT

Scope: Interpretation of rapid and advanced microbiological test results remains nonstandardized, with no existing reference guidelines. This study aimed to analyse the existing evidence and provide expert guidance on the use of these techniques in critically ill patients.
Methods: A Delphi consensus process was conducted by a multidisciplinary panel of experts, including microbiologists, infectious disease specialists, intensivists, surgeons, and pulmonologists. Sixteen prioritized key questions were addressed via literature reviews and two Delphi rounds. Consensus was reached when 70% of the responses showed strong agreement.
Questions addressed by consensus and recommendations: Consensus was reached for all 16 statements. The key findings include the importance of interpreting rapid microbiological test results within a specific clinical context; the need for concurrent standard culture examinations alongside rapid tests to ensure the detection of all pathogens; the clinical usefulness of turnaround times <24 hours for rapid techniques; and the benefits of rapid diagnostics, particularly in severe sepsis and other severe infections. Specific recommendations were made regarding the use of rapid tests in various clinical settings (critically ill patients with suspected infection, pneumonia, and ventilator-associated pneumonia). The panel found insufficient evidence to support the routine use of digital polymerase chain reaction in various infection scenarios and concluded that clinical bioinformatics expertise is essential in microbiology laboratories that use advanced technologies. The panel also highlighted the need for basic clinician training to interpret data generated using advanced microbiological techniques. This consensus provides guidance for the appropriate use of rapid and advanced microbiological techniques for critically ill patients. However, the standardization of testing settings, interpretations, and cost-effectiveness analyses of different approaches requires further investigation. Robust preanalytical workflows and multidisciplinary clinical bioinformatics expertise are crucial for the effective implementation and interpretation of advanced techniques. **Vittorio Sambri, Clin Microbiol Infect 2025;31:1993**

Scope

Although the accurate measurement of the incidence of sepsis remains challenging, it affects approximately 48 000 000 to 49 000 000 people globally each year, with 11 million associated deaths. This accounts for 19.7% of the global mortality [1].

Standard operating procedures for the rapid identification of patients with sepsis and potential pathogens include microbiological culture tests [2]. Standard microbiological tests take an average of 3 days to produce results, a delay that often necessitates the use of empirical therapies, with the risk of administering ineffective treatments. The use of antimicrobials contributes to the emergence of antibiotic resistance, making it crucial to limit the number and spectrum of antibiotics, particularly for sepsis and septic shock. This is particularly crucial in empirical therapy, in which broad-spectrum agents are administered before pathogen identification [2,3].

Recently, a wide array of rapid molecular and phenotypic tests has revolutionized microbiological diagnostics, significantly reducing waiting times [4]. Early identification of the pathogen and any resistance mechanism allows the initiation of targeted anti-infective therapy hours or even days prior. Next generation sequencing (NGS) techniques are increasingly being used and implemented in clinical microbiology and reference laboratories, which rely heavily on bioinformatics for data processing, analysis, interpretation, and communication. Advanced microbiological tools are gradually being integrated into clinical practice, thereby expanding their diagnostic and therapeutic possibilities [5]. Digital PCR (dPCR) techniques are also being used in clinical practice [6]. However, the settings, patient populations, and interpretations of rapid and advanced microbiological test results remain non-standardized, and no reference guidelines exist.

Context

We aimed to analyse the literature on this subject and to provide microbiologists, clinicians, laboratory professionals, and the scientific community with evidence-based guidance.

Methods

A group of five proponents—V.A., F.C., F.C., E.R., and V.S.—suggested the formation of an expert panel comprising microbiologists (G.M.R., M.S., S.S., T.P., P.B., A.R., C.F., and N.M.), infectious disease specialists (M.B., F.G.D.R., M.B., M.F., I.G., M.G., A.O., A.R., C. T., M.T., P.V., and G.V.), intensivists (B.V., G.D.P., M.G., G.G., O.P., E.G. B., L.G., and D.P.S.), surgeons (F.C. and M.S.), and a pulmonologist (V.P.) with demonstrated expertise, clinical experience, research, and dissemination activities in the field of microbiological diagnostics for critically ill patients.

The initiative received endorsements from leading scientific societies relevant to the topic and the Italian Ministerial Committee for Combating Antimicrobial Resistance.

The key questions proposed by the promotion group were reviewed by an expert panel through a series of Delphi rounds and collaborative evaluations [7–9].

The expert panel evaluated 28 questions during the first round of the Delphi consensus, resulting in the approval of 13 questions, of which 15 were excluded (Table 1). Of these 15 questions, seven were reformulated and submitted for evaluation in a second Delphi round. Three out of seven reformulated questions were subsequently approved and added to the 13 previously approved questions, for a total of 16 key questions (Table 2). Ultimately, 16 questions were presented, generating 16 corresponding statements (Tables 3 and 4).

Subsequently, the expert panel selected a group of reviewers to conduct a systematic literature review.

The literature review was made available to all panel members, organized according to questions, and standardized into summary tables.

The expert panel convened in person for a 2-day consensus conference, discussing all 16 questions and generating the statements. The meeting was held at Rocca delle Caminate (FC, Italy) on 28 October, 2024 and 29 October, 2024.

Consensus was achieved when $\geq$70% of responses were in strong agreement (7–9 on the nine-point Likert scale), and strong disagreement (1–3) was <15%.

The rationale for each statement is provided in the Supplementary Materials.

Recommendations

An agreement was reached for 100% of the statements.

Key Question 1. How should the results be utilized in critically ill patients undergoing rapid microbiological tests?

Statement 1. In critically ill patients with suspected infections, rapid microbiological tests should be interpreted within a specific clinical context and evaluated using timely diagnostic and antimicrobial stewardship programmes to maximize their clinical impact.

Strongly agree (7–9), 94.1%; strongly disagree (1–3), 5.9%

Quality of Evidence (QoE): Low

Key Question 2. In critically ill patients undergoing rapid microbiological testing (blood and other site specimens), is the concurrent execution of standard culture examinations preferred over rapid microbiological testing alone in terms of the pathogen identification rate?

Statement 2. In critically ill patients undergoing rapid microbiological testing (blood and other site samples), the concurrent execution of standard culture examinations is preferred over rapid microbiology alone in terms of the pathogen identification rate, considering that the currently available rapid microbiological methods do not detect all pathogens. Moreover, standard cultures allow phenotypic antimicrobial susceptibility testing and collection of isolates for epidemiological and infection control purposes.

Strongly agree (7–9), 93.4%; strongly disagree (1–3), 3.3%

QoE: Low

Key Question 3. Which turnaround times (TATs) are clinically useful for reports based on rapid techniques? Is there an upper limit that reduces the clinical value of the test?

Statement 3. In critically ill patients undergoing rapid microbiological testing, evidence from the reviewed studies suggests that TATs <24 hours can maximize the clinical utility of rapid microbiological techniques by optimizing antimicrobial therapy. The panel suggests that the total TATs (including the preanalytical, analytical, and postanalytical phases) should be as close as possible to the actual analytical phase of the available assays.

Strongly agree (7–9), 75.7%; strongly disagree (1–3), 12.1%

QoE: Low

Key Question 4. In critically ill patients with suspected infections, are blood cultures (BCs) with rapid microbiological tests preferred over standard microbiological tests in terms of the pathogen identification time, cure rate, mortality, intensive care unit (ICU) length of stay, and overall hospital length of stay?

Statement 4. For critically ill patients with severe clinical presentation and positive BCs, the panel suggests using rapid tests to reduce the time to optimal antimicrobial therapy.

Strongly agree (7–9), 88.3%; strongly disagree (1–3), 2.9%

QoE: High

Key Question 5. In critically ill patients with community-acquired pneumonia (CAP), is rapid microbiological testing of respiratory samples preferred over standard microbiological tests in terms of the pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and duration of mechanical ventilation?

Statement 5. In critically ill patients with severe CAP who present with clinical and radiological risk factors for the failure of standard therapy, the panel suggests the use of rapid microbiological testing (PCR-based syndromic panels) for lower respiratory samples, in addition to standard microbiological testing, to improve pathogen detection rates, provide early results, and enhance precision in antimicrobial management.

Strongly agree (7–9), 88.1%; strongly disagree (1–3), 0.0%

QoE: Moderate

Key Question 6. In critically ill patients with ventilator-associated pneumonia (VAP), is rapid microbiological testing of respiratory samples preferred over standard microbiological testing in terms of the pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and duration of mechanical ventilation?

Statement 6. In critically ill patients with VAP and ventilated hospital-acquired pneumonia, the panel suggests the use of rapid microbiological techniques (PCR-based syndromic panels) in addition to standard microbiological testing of lower respiratory samples to increase pathogen detection rates and improve the appropriateness of antibiotic treatment.

Strongly agree (7–9), 100.0%; strongly disagree (1–3), 0.0%

QoE: Moderate

Key Question 7. In patients undergoing rapid microbiological testing of lower respiratory tract samples, is bronchoalveolar lavage (BAL) preferred over bronchial aspirate (BA) in terms of the pathogen identification rate?

Statement 7. In critically ill patients with lower respiratory tract infections, the panel suggests that the current evidence is insufficient to recommend BAL over BA for rapid microbiological testing; however, deep respiratory samples should be considered more appropriate than endotracheal aspirates and sputum.

Strongly agree (7–9), 90.7%, strongly disagree (1–3), 3.1%

QoE: Low

Key Question 8. In critically ill patients with pneumonia (CAP and VAP), are dPCR tests for respiratory samples indicated in association with standard microbiological tests in terms of pathogen identification rate and time?

Statement 8. In critically ill patients with pneumonia, including CAP and VAP, there is currently insufficient evidence to support the use of dPCR in terms of TATs and identification rates. Furthermore, dPCR can be used to assess antimicrobial susceptibility profiles and pathogen loads.

Strongly agree (7–9), 96.8%; strongly disagree (1–3), 0.0%

QoE: Low

Table 1
Proposed research questions and agreement

Proposed research questions and agreement	# Round	% Agreement
1. In critically ill patients with suspected infection, are blood cultures (BCs) with fast microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit (ICU) length of stay, and hospital length of stay?	1	93,3
2. In critically ill patients with community-acquired pneumonia (CAP), is rapid microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and mechanical ventilation duration?	1	73,4
3. In critically ill patients with infection-related ventilator-associated complications (iVAC), is rapid microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and mechanical ventilation duration?	1	83,3
4. In patients undergoing fast microbiology testing from lower respiratory tract samples, is a bronchoalveolar lavage preferred over a bronchial aspirate in terms of pathogen identification rate?	1	86,6
5. In patients with suspected community-acquired central nervous system (CNS) infection, is rapid microbiology testing of cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	86,7
6. In patients with suspected postneurosurgical CNS infection, is rapid microbiological testing of CSF indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	73,3
7. In patients with suspected abdominal infection, is rapid microbiologiy testing of abdominal samples indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	43,3
8. In critically ill patients undergoing fast microbiology tests, how should the results be utilized?	1	86,7
9. In critically ill patients undergoing rapid microbiology testing (BCs and other site samples), is concurrent execution of standard culture examination indicated over rapid microbiology testing alone in terms of pathogen identification rate?	1	90
10. In critically ill patients undergoing fast or advanced microbiology testing is sepsis biomarker assay indicated over not using biomarkers in terms of sepsis identification and antibiotic duration?	1	80
11. In critically ill patients with suspected infection, is the processing of BCs with next generation sequencing (NGS) microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	66,6
12. In patients with CAP is NGS microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and mechanical ventilation duration?	1	43,3
13. In critically ill patients with iVAC is NGS microbiological testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, mechanical ventilation duration?	1	63,3
14. In patients with suspected community-acquired CNS infection is NGS microbiological testing of CSF indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	40,1
15. In patients with suspected postneurosurgical CNS infection (P) is NGS microbiological testing of CSF (I) indicated over standard microbiology tests (C) in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	60
16. In patients undergoing advanced microbiology testing from lower respiratory tract samples, is a bronchoalveolar lavage or bronchial aspirate preferred?	1	83,4
17. In patients with a suspected abdominal infection is NGS testing of abdominal samples indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	53,3
18. What are the hypothesized analytical advantages and disadvantages of NGS and digital PCR (dPCR) techniques?	1	66,7
19. In which clinical/epidemiological settings can dPCR play a role?	1	56,7
20. In critically ill patients with suspected infection, is the processing of BCs with dPCR microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay?	1	63,3
21. In patients with CAP is dPCR microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and mechanical ventilation duration?	1	46,7
22. In critically ill patients with iVAC is dPCR microbiological testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, mechanical ventilation duration?	1	56,7
23. In patients with a suspected community-acquired CNS infection is dPCR microbiological testing of CSF indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	63,4
24. In patients with suspected postneurosurgical CNS infection is dPCR microbiological testing of CSF indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	56,7
25. In patients with suspected abdominal infection is dPCR testing of abdominal samples indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?	1	46,7
26. Which turnaround times are clinically useful for reports based on advanced techniques? Is there an over limit that reduces the clinical value of the test?	1	80
27. What preanalytical workflow guarantees are considered essential for these technologies? Centralization or spread?	1	93,3
28. What clinical-bioinformatic expertise should be available within the network? Training (basic) for clinicians on the clinical value of the analytical data generated by advanced microbiology?	1	80

Key Question 9. In critically ill patients undergoing advanced microbiological testing of lower respiratory tract samples, should BAL be preferred over BA?

Statement 9. In critically ill patients with clinical indications for advanced microbiological testing of lower respiratory tract

Table 2
Reformulated Delphi questions

Reformulated Delphi questions	# Round	% Agreement
In critically ill patients with suspected infection, is the processing of respiratory samples (bronchoalveolar lavage) with digital PCR (dPCR) microbiology tests indicated in association standard microbiology tests in terms of pathogen identification rate and/or time?	2	88
In critically ill patients with suspected infection, is the processing of blood cultures with next generation sequencing (NGS) microbiology tests indicated over standard microbiology tests in terms of pathogen identification rate?	2	43.3
In critically ill patients with suspected infection, is the processing of blood cultures with dPCR microbiology tests indicated over standard microbiology tests in terms of pathogen identification rate and/or time?	2	60
In critically ill patients suffering from pneumonia (both community-acquired or Infection-Related Ventilator-Associated Complication) are dPCR microbiology tests of respiratory samples indicated in association with standard microbiology tests in terms of pathogen identification rate and/or time?	2	76%
In critically ill patients suffering from pneumonia (both community-acquired or infection-related ventilator-associated complication) are next generation sequencing microbiology tests of respiratory samples indicated over standard microbiology tests in terms of pathogen identification rate and/or time?	2	46.7
In patients with suspected central nervous system infection (both community-acquired are postneurosurgical) are next generation sequencing microbiology tests of cerebrospinal fluid indicated over standard microbiology tests in terms of pathogen identification rate and/or time?	2	84
In patients with suspected central nervous system infection (both community-acquired and postneurosurgical) are dPCR microbiological testing of cerebrospinal fluid indicated over standard microbiology tests in terms of pathogen identification rate and/or time?	2	80

Table 3
Final research questions

Q#	Final research questions
1	In critically ill patients undergoing rapid microbiology tests, how should the results be utilized?
2	In critically ill patients undergoing rapid microbiology testing (blood and other site specimens), is the concurrent execution of standard culture examinations indicated over rapid microbiology testing alone in terms of pathogen identification rate?
3	Which turnaround times (TATs) are clinically useful for reports based on rapid techniques? Is there an upper limit that reduces the clinical value of the test?
4	In critically ill patients with suspected infection, are blood cultures with rapid microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit (ICU) length of stay, and overall hospital length of stay?
5	In critically ill patients with community-acquired pneumonia, is rapid microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, mechanical ventilation duration?
6	In critically ill patients with ventilator-associated pneumonia, is rapid microbiology testing of respiratory samples preferred over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, ICU length of stay, hospital length of stay, and duration of mechanical ventilation?
7	In patients undergoing rapid microbiology testing from lower respiratory tract samples, is a bronchoalveolar lavage preferred over a bronchial aspirate in terms of rate of pathogen identification?
8	In critically ill patients suffering from pneumonia (both community-acquired and ventilator-associated pneumonia), are digital PCR tests of respiratory samples indicated in association with standard microbiology tests in terms of pathogen identification rate and time?
9	In critically ill patients undergoing advanced microbiology testing of lower respiratory tract samples, should bronchoalveolar lavage be preferred over bronchial aspirate?
10	In patients with suspected community-acquired central nervous system (CNS) infection, is rapid microbiology testing of cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, hospital length of stay?
11	In patients with suspected healthcare-associated CNS infection, is rapid microbiological testing of CSF indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and the hospital length of stay?
12	In patients with suspected CNS infection (both community-acquired and healthcare-associated), are next generation sequencing microbiology tests of CSF indicated over standard microbiology tests in terms of pathogen identification rate and time?
13	In patients with suspected CNS infection (both community-acquired and postneurosurgical), is digital PCR microbiological testing of CSF indicated over standard microbiology tests in terms of pathogen identification rate and time?
14	In critically ill patients undergoing rapid or advanced microbiology testing, is a sepsis biomarker assay indicated compared with not using biomarkers in terms of sepsis identification and the duration of antibiotic treatment?
15	What essential preanalytical workflow guarantees are necessary for rapid and advanced technologies: centralization or spread?
16	What clinical-bioinformatic expertise should be available within the network? Should there be basic training for clinicians on the interpretation and clinical value of the analytical data generated by advanced microbiology?

samples, there is insufficient evidence to suggest either BAL or BA for diagnosing lower respiratory tract infections when advanced microbiological techniques, such as mNGS, are applied.

However, in critically ill patients undergoing advanced microbiological testing of lower respiratory tract samples, the panel suggests the use of BAL when clinically feasible and in alignment with the logistical constraints and expertise of operators.

Strongly agree (7–9), 82.9%; strongly disagree (1–3), 5.8%.

QoE: Low

Key Question 10. In patients with suspected community-acquired central nervous system (CNS) infections, is rapid microbiological testing of the cerebrospinal fluid (CSF) preferred over standard microbiological tests in terms of the pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?

Statement 10. In critically ill patients with suspected community-acquired meningitis and encephalitis, the panel suggests performing rapid microbiological testing (PCR-based syndromic panels) of the CSF together with standard culture- and molecular-based assays to increase the pathogen detection rate and reduce the time to appropriate antimicrobial treatment.

Strongly agree (7–9), 87.9%; strongly disagree (1–3), 0.0%

QoE: Low

Key Question 11. In patients with suspected healthcare-associated CNS infections (HCA-CNS-I), is rapid microbiological testing of the CSF preferred over standard microbiological tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, ICU length of stay, and hospital length of stay?

Statement 11. In critically ill patients with suspected HCA-CNS-I, the panel advises against the use of commercially available rapid microbiological testing (PCR-based syndromic panels) of the CSF.

Strongly agree (7–9), 96.9%; strongly disagree (1–3), 3.1%

QoE: Not assessable. (We did not find any relevant study specifically assessing the study question.)

Key Question 12. In patients with suspected CNS infection (both community-acquired and healthcare-associated), are NGS

Table 4
Final statements

S#	Final statements	SA (7-9) %	SD (1–3) %	QoE
1	In critically ill patients with suspected infections, rapid microbiology tests should be interpreted within the specific clinical context and evaluated through a timely diagnostic and antimicrobial stewardship programme to maximize their clinical impact.	94.1	5.9	Low
2	In critically ill patients undergoing rapid microbiology testing (blood and other site samples), the concurrent execution of standard culture examinations is indicated over rapid microbiology alone in terms of pathogen identification rate, considering that currently available rapid microbiology methods are not able to detect all pathogens. Moreover, standard cultures allow phenotypic antimicrobial susceptibility testing and the collection of isolates for epidemiological and infection control purposes.	93.4	3.3	Low
3	In critically ill patients undergoing rapid microbiological testing, evidence from the reviewed studies suggests that turnaround times (TATs) under 24 hours can maximize the clinical utility of rapid microbiology techniques by optimizing antimicrobial therapy. The panel suggests that the total TATs (including preanalytical, analytical, and postanalytical phases) should be as close as possible to the actual analytical phase of the available assays.	75.7	12.1	Low
4	For critically ill patients with severe clinical presentations and positive blood cultures, the panel suggests using rapid tests to reduce the time to optimal antimicrobial therapy.	88.3	2.9	High
5	In critically ill patients with severe community-acquired pneumonia (CAP), presenting clinical and radiological risk factors for failure of standard therapy, the panel suggests the use of rapid microbiology testing (PCR-based syndromic panels) on lower respiratory samples, in addition to standard microbiological testing, to improve pathogen detection rates, provide earlier results, and enhance precision in antimicrobial management.	88.1	0	Moderate
6	In critically ill patients with ventilator-associated pneumonia (VAP) and ventilated hospital-acquired pneumonia (vHAP), the panel suggests considering rapid microbiology techniques (PCR-based syndromic panels) in addition to standard microbiological testing on lower respiratory samples, to increase pathogen detection rates and improve appropriateness in antibiotic treatment.	100	0	Moderate
7	In critically ill patients with lower respiratory tract infections, the panel suggests that current evidence is insufficient to recommend bronchoalveolar lavage (BAL) over bronchial aspirate for rapid microbiology testing; however, deep respiratory samples should be considered more appropriate than endotracheal aspirates and sputum.	90.7	3.1	Low
8	In critically ill patients with pneumonia, including both community-acquired and ventilator-associated pneumonia, there is currently not enough evidence to support the use of digital PCR (dPCR) in terms of TATs and identification rates. Furthermore, dPCR could be applied to the assessment of antimicrobial susceptibility profiles and pathogen load.	96.8	0	Low
9	In critically ill patients with clinical indications to advanced microbiology testing from lower respiratory tract samples, there is insufficient evidence to suggest either BAL or bronchial aspirate for diagnosing lower respiratory tract infections when advanced microbiological techniques are applied, such as metagenomic next generation sequencing. However, in critically ill patients undergoing advanced microbiology testing on lower respiratory tract samples, the panel suggests using BAL when clinically feasible and in alignment with the logistical constraints and expertise of the operators.	82.9	5.8	Low
10	In critically ill patients with suspected community-acquired meningitis or encephalitis, the panel suggests performing rapid microbiology testing (PCR-based syndromic panels) of cerebrospinal fluid, together with standard culture- and molecular-based assays, to increase pathogen detection rate and reduce the time to appropriate antimicrobial treatment.	87.9	0	Low
11	In critically ill patients with suspected healthcare-associated central nervous system (CNS) infections, the panel advices against the use of commercially available rapid microbiology testing (PCR-based syndromic panels) of cerebrospinal fluid.	96.9	3.1	NA
12	In critically ill patients with suspected CNS infection (both community-acquired and healthcare-associated), with negative standard diagnostic tests, the panel suggests the use of next generation sequencing microbiology tests on cerebrospinal fluid, brain abscesses and biopsy samples in addition to standard microbiology tests to enhance microbiological profiling. The panel does not provide any indication about specific different sequencing technologies.	94.2	0	Low
13	In patients with suspected CNS infections, including both community-acquired and healthcare-associated cases, there is insufficient evidence to suggest that dPCR on cerebrospinal fluid offers advantages over standard microbiology tests in improving pathogen identification rates or reducing TATs.	94	0	Low
14	In critically ill patients undergoing rapid or advanced microbiology testing, evidence is insufficient to generate recommendations on whether a sepsis biomarker assay is indicated over not using biomarkers in terms of sepsis identification and the duration of antibiotic treatment.	73.5	14.7	Low
15	There is insufficient evidence to determine if a centralized or decentralized workflow is indicated. However, in critically ill patients with infection, the panel suggests that decentralization of rapid microbiology techniques (spoke laboratories) could be considered, in line with logistical feasibility, sustainability, and staff capabilities, to optimize pathogen identification times and initiation of appropriate therapy, while advanced techniques should be performed in highly specialized laboratories. In all cases, preanalytical guarantees are crucial for ensuring the reliability of advanced microbiology technologies.	78.2	6.2	Low
16	There is enough evidence to establish that clinical-bioinformatic expertise is essential for microbiology laboratories using advanced technologies. Additionally, training for clinicians on the clinical value of the analytical data generated by advanced microbiology would improve patient care and public health outcomes.	90.9	3	Low

QoE, quality of evidence.

microbiological tests of the CSF preferred over standard microbiological tests in terms of the pathogen identification rate and time?

Statement 12. In critically ill patients with suspected CNS infection (both community-acquired and healthcare-associated) and negative standard diagnostic tests, the panel suggests the

use of NGS microbiological tests on CSF, brain abscesses, and biopsy samples in addition to standard microbiological tests to enhance microbiological profiling. The panel does not provide any indication for the use of specific sequencing technologies.

Strongly agree (7–9), 94.2%; strongly disagree (1–3), 0.0%

QoE: Low

Key Question 13. In patients with suspected CNS infection (both community-acquired and post-neurosurgical), is dPCR microbiological testing of the CSF preferred over standard microbiological tests in terms of the pathogen identification rate and time?

Statement 13. In patients with suspected CNS infections, including both community-acquired and healthcare-associated cases, there is insufficient evidence to suggest that dPCR of the CSF offers advantages over standard microbiological tests in improving pathogen identification rates or reducing TATs.

Strongly agree (7–9), 94.0%; strongly disagree (1–3), 0.0%

QoE: Low

OMITTED Key Question 14. In critically ill patients undergoing rapid or advanced microbiological testing, is a sepsis biomarker assay preferred over not using biomarkers in terms of sepsis identification and duration of antibiotic treatment?

Statement 14. In critically ill patients undergoing rapid or advanced microbiological testing, evidence is insufficient to generate recommendations on whether a sepsis biomarker assay is preferred over not using biomarkers for sepsis identification and the duration of antibiotic treatment.

Strongly agree (7–9), 73.5%; strongly disagree (1–3), 14.7%

QoE: Low

Key Question 15. What essential preanalytical workflow guarantees are necessary for rapid and advanced technologies: centralization or spread?

Statement 15. There is insufficient evidence to determine whether a centralized or decentralized workflow is indicated. However, in critically ill patients with infection, the panel suggests that decentralization of rapid microbiological techniques (spoke laboratories) could be considered, in line with logistical feasibility, sustainability, and staff capabilities, to optimize pathogen identification times and initiate appropriate therapy, whereas advanced techniques should be performed in highly specialized laboratories (hub). In all cases, maintaining preanalytical quality is essential to ensure the reliability of advanced microbiological diagnostics.

Strongly agree (7–9), 78.2%; strongly disagree (1–3), 6.2%

QoE: Low

Key Question 16. What clinical bioinformatics expertise should be available in the network? Should there be basic training for clinicians on the interpretation and clinical value of analytical data generated using advanced microbiological techniques?

Statement 16. There is sufficient evidence to establish that expertise in clinical bioinformatics is essential for microbiology laboratories that use advanced technologies. Additionally, training clinicians on the clinical value of analytical data generated using advanced microbiological techniques would improve patient care and public health outcomes.

Strongly agree (7–9), 90.9%; strongly disagree (1–3), 3.0%

QoE: Low

Discussion

Owing to the lack of reference guidelines, the settings, patient populations, and interpretations of rapid and advanced microbiological test results remain nonstandardized. In this study, we aimed to analyse the literature on this subject and to lay a strong scientific foundation for the use of advanced microbiological tools in critically ill patients. The strength of this study lies in its development by a multidisciplinary panel of microbiologists, infectious disease specialists, intensivists, surgeons, and pulmonologists. The exceptionally high level of agreement achieved was likely due to the collaborative approach adopted during the 2-day consensus meeting in which experts generated shared statements.

This position paper should be a clinical guide for clinicians and microbiologists for the diagnosis and treatment of critically ill patients with severe infections. Up to now there are not in the current literature guidelines or position papers on this topic.

In the present position paper, there are nine statements with >90% agreement. Especially, the panel achieved 100% agreement that in critically ill patients with ventilator-associated pneumonia and ventilated hospital-acquired pneumonia, enhanced pathogen detection rates and appropriateness in antibiotic treatment can be improved using rapid microbiology techniques (PCR-based syndromic panels) and standard microbiological testing on lower respiratory samples.

Moreover, the panel for the same category of patients had a very high agreement (96.8%) with regard to the use of dPCR in terms of achieving a precise and rapid diagnosis of pneumonia, including the assessment of the bacterial load.

In statement number 12, the panel achieved high agreement (94.2%) in favour of the use of NGS on CNS biological samples when standard microbiology tests are negative in patients with suspected CNS infection (both community-acquired and healthcare-associated).

The steering committee, following the criticism raised by one of the reviewers, decided to omit statement number 14 because it is out of the scope of the current position paper. Biomarkers are not part of rapid microbiological testing, even if they are highly significant clinical drivers for treatment decisions.

For topics where the current evidence does not allow for specific recommendations, particularly concerning respiratory sampling materials for rapid microbiological methods, application of advanced microbiological techniques, and the role of biomarkers in managing patients undergoing rapid and advanced microbiological testing, a call for research is warranted.

Note on the use of this consensus

This consensus aims to support clinicians' decision-making in the management of critically ill patients requiring rapid and advanced microbiological diagnostics. These statements are intended to assist clinical judgements in providing timely personalized therapy. Given the limited number of high-quality studies in this field, we used a modified Delphi method to engage national experts.

The recommendations herein are advisory, based on the best available evidence and expert consensus, and they do not establish a legal standard of care. Alternate approaches may also be valid within accepted practice standards. Ultimately, the responsibility for patient outcomes lies with the treating clinicians and not with the consensus group. Importantly, close collaboration across medical specialties (i.e. a multidisciplinary approach) is essential to improve outcomes in critically ill patients, both in the initial acute phase and throughout prolonged care.

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Transparency declaration

Potential conflict of interest

The authors declare that they have no conflicts of interest.

Financial report

No external funding was received for the study.

Acknowledgements

We would like to thank Dr. Emanuele Russo, who first had the idea to organize this consensus. Sadly, he passed away suddenly and far too soon, leaving behind the love and esteem of his family and colleagues. The authors respectfully dedicate this consensus to his memory.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.08.022.

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