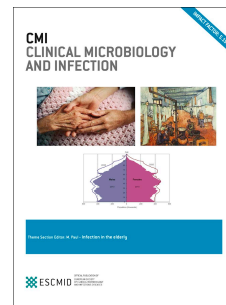


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The Role of Rapid and Advanced Microbiological Methods in Critical Care: 2025  
EMANUELE RUSSO Delphi Consensus

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8

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## 181 **Keywords**

182 Delphi, consensus, microbiology, rapid, critical care

183

184

## 185 **ABSTRACT**

### 186 **Scope**

187 Interpretation of rapid and advanced microbiological test

188 results remains nonstandardized, with no existing reference

189 guidelines. This study aimed to analyze the existing evidence

190 and provide expert guidance on the use of these techniques in

191 critically ill patients.

### 192 **Methods**

193 A Delphi consensus process was conducted by a

194 multidisciplinary panel of experts, including microbiologists,

195 infectious disease specialists, intensivists, surgeons, and

196 pulmonologists. Sixteen prioritized key questions were

197 addressed via literature reviews and two Delphi rounds.

198 Consensus was reached when 70% of the responses showed

199 strong agreement.

### 200 **Questions addressed by consensus and Recommendations**

201 Consensus was reached for all 16 statements. The key findings  
202 include the importance of interpreting rapid microbiological  
203 test results within a specific clinical context; the need for  
204 concurrent standard culture examinations alongside rapid tests  
205 to ensure the detection of all pathogens; the clinical usefulness  
206 of turnaround times <24 h for rapid techniques; the benefits of  
207 rapid diagnostics, particularly in severe sepsis and other severe  
208 infections. Specific recommendations were made regarding the  
209 use of rapid tests in various clinical settings (critically ill  
210 patients with suspected infection, pneumonia, and ventilator-  
211 associated pneumonia). The panel found insufficient evidence  
212 to support the routine use of digital polymerase chain reaction  
213 in various infection scenarios and concluded that clinical  
214 bioinformatics expertise is essential in microbiology  
215 laboratories that use advanced technologies. The panel also  
216 highlighted the need for basic clinician training to interpret data  
217 generated using advanced microbiological techniques.  
218 This consensus provides guidance for the appropriate use of  
219 rapid and advanced microbiological techniques for critically ill  
220 patients. However, the standardization of testing settings,  
221 interpretations, and cost-effectiveness analyses of different  
222 approaches require further investigation. Robust preanalytical  
223 workflows and multidisciplinary clinical bioinformatics  
224 expertise are crucial for the effective implementation and  
225 interpretation of advanced techniques.

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**235 FULL DOCUMENT**

236

**237 Scope**

238 Although the accurate measurement of the incidence of sepsis  
239 remains challenging, it affects approximately 48–49,000,000  
240 people globally each year, with 11 million associated deaths.

241 This accounts for 19.7% of the global mortality [1].

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

253 Recently, a wide array of rapid molecular and phenotypic tests  
254 has revolutionized microbiological diagnostics, significantly  
255 reducing waiting times [4]. Early identification of the pathogen  
256 and any resistance mechanism allows the initiation of targeted  
257 anti-infective therapy hours or even days prior. Next-generation  
258 sequencing (NGS) techniques are increasingly being used and  
259 implemented in clinical microbiology and reference  
260 laboratories, which rely heavily on bioinformatics for data  
261 processing, analysis, interpretation, and communication.

262 Advanced microbiological tools are gradually being integrated  
263 into clinical practice, thereby expanding their diagnostic and  
264 therapeutic possibilities [5]. Digital polymerase chain reaction  
265 (dPCR) techniques are also being used in clinical practice [6].  
266 However, the settings, patient populations, and interpretations  
267 of rapid and advanced microbiological test results remain  
268 nonstandardized, and no reference guidelines exist.

**269 Context**

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

273

**274 Methods**

275 A group of five proponents—VA, FC, ER, and VS—  
276 suggested the formation of an expert panel comprising  
277 microbiologists (GMR, MS, SS, TP, PB, AR, CF, and NM),  
278 infectious disease specialists (MB, FGDR, MB, MF, IG, MG,  
279 AO, AR, CT, MT, PV, GV), intensivists (BV, GDP, MG, GG,  
280 OP, EGB, LG, and DPS), surgeons (FC, MS), and a  
281 pulmonologist (VP) with demonstrated expertise, clinical  
282 experience, research, and dissemination activities in the field of  
283 microbiological diagnostics for critically ill patients.

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

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

290 The expert panel evaluated 28 questions during the first round  
291 of the Delphi consensus, resulting in the approval of 13  
292 questions, of which 15 were excluded (Table 1). Of these 15  
293 questions, 7 were reformulated and submitted for evaluation in  
294 a second Delphi round. Three out of 7 reformulated questions  
295 were subsequently approved and added to the 13 previously  
296 approved questions, for a total of 16 key questions (Table 2).  
297 Ultimately, 16 questions were presented, generating 16  
298 corresponding statements (Tables 3 and 4).

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

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

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

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

311 The rationale for each statement is provided in the  
312 supplementary materials.

313

#### 314 **Recommendations**

315 Agreement was reached for 100% of the statements.

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

318 **Statement 1.** In critically ill patients with suspected infections,  
319 rapid microbiological tests should be interpreted within a  
320 specific clinical context and evaluated using timely diagnostic  
321 and antimicrobial stewardship programs (ASPs) to maximize  
322 their clinical impact.

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

324 Quality of Evidence (QoE): Low

325

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

331 **Statement 2.** In critically ill patients undergoing rapid  
332 microbiological testing (blood and other site samples), the  
333 concurrent execution of standard culture examinations is  
334 preferred over rapid microbiology alone in terms of the

335 pathogen identification rate, considering that the currently  
336 available rapid microbiological methods do not detect all  
337 pathogens. Moreover, standard cultures allow phenotypic  
338 antimicrobial susceptibility testing and collection of isolates for  
339 epidemiological and infection control purposes.

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

341 QoE: Low

342

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

346 **Statement 3.** In critically ill patients undergoing rapid  
347 microbiological testing, evidence from the reviewed studies  
348 suggests that TATs <24 h can maximize the clinical utility of  
349 rapid microbiological techniques by optimizing antimicrobial  
350 therapy. The panel suggests that the total TATs (including the  
351 preanalytical, analytical, and post-analytical phases) should be  
352 as close as possible to the actual analytical phase of the  
353 available assays.

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

355 QoE: Low

356

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

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

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

367 QoE: High

368

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

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

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

384 QoE: Moderate

385

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

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

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

399 QoE: Moderate

400

401 **Key Question 7.** In patients undergoing rapid microbiological  
402 testing of lower respiratory tract samples, is bronchoalveolar

403 lavage (BAL) preferred over bronchial aspirate (BA) in terms  
404 of the pathogen identification rate?

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

411 Strongly agree (7–9), 90.7%, strongly disagree (1–3), 3.1%  
412 QoE: Low

413  
414 **Key Question 8.** In critically ill patients with pneumonia (CAP  
415 and VAP), are dPCR tests for respiratory samples indicated in  
416 association with standard microbiological tests in terms of  
417 pathogen identification rate and time?

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

423 Strongly agree (7–9), 96.8%; strongly disagree (1–3), 0.0%  
424 QoE: Low

425  
426 **Key Question 9.** In critically ill patients undergoing advanced  
427 microbiological testing of lower respiratory tract samples,  
428 should BAL be preferred over BA?

429 **Statement 9.** In critically ill patients with clinical indications  
430 for advanced microbiological testing of lower respiratory tract  
431 samples, there is insufficient evidence to suggest either BAL or  
432 BA for diagnosing lower respiratory tract infections when  
433 advanced microbiological techniques, such as mNGS, are  
434 applied.

435 However, in critically ill patients undergoing advanced  
436 microbiological testing of lower respiratory tract samples, the

437 panel suggests the use of BAL when clinically feasible and in  
438 alignment with the logistical constraints and expertise of  
439 operators.

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

441 QoE: Low

442

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

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

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

457 QoE: Low

458

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

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

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

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

472

473 **Key Question 12.** In patients with suspected CNS infection  
474 (both community-acquired and healthcare-associated), are NGS  
475 microbiological tests of the CSF preferred over standard  
476 microbiological tests in terms of the pathogen identification  
477 rate and time?

478 **Statement 12.** In critically ill patients with suspected CNS  
479 infection (both community-acquired and healthcare-associated)  
480 and negative standard diagnostic tests, the panel suggests the  
481 use of NGS microbiological tests on CSF, brain abscesses, and  
482 biopsy samples in addition to standard microbiological tests to  
483 enhance microbiological profiling. The panel does not provide  
484 any indication for the use of specific sequencing technologies.

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

486 QoE: Low

487

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

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

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

499 QoE: Low

500

501 **OMITTED Key Question 14.** In critically ill patients  
502 undergoing rapid or advanced microbiological testing, is a  
503 sepsis biomarker assay preferred over not using biomarkers in

504 terms of sepsis identification and duration of antibiotic  
505 treatment?

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

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

512 QoE: Low

513

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

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

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

529 QoE: Low

530

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

536 **Statement 16.** There is sufficient evidence to establish that  
537 expertise in clinical bioinformatics is essential for microbiology

538 laboratories that use advanced technologies. Additionally,  
539 training clinicians on the clinical value of analytical data  
540 generated using advanced microbiological techniques would  
541 improve patient care and public health outcomes.  
542 Strongly agree (7–9), 90.9%; strongly disagree (1–3), 3.0%  
543 QoE: Low

544

## 545 **DISCUSSION**

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

558 This position paper should be a clinical guide for clinicians and  
559 microbiologists for the diagnosis and treatment of critically ill  
560 patients with severe infections. Up to now there are not in the  
561 current literature guidelines or position papers on this topic.  
562 In the present position paper, there are nine statements with  
563 more than 90% agreement. Especially, the panel achieved  
564 100% agreement that in critically ill patients with ventilator-  
565 associated pneumonia and ventilated hospital-acquired  
566 pneumonia, enhanced pathogen detection rates and  
567 appropriateness in antibiotic treatment can be improved using  
568 rapid microbiology techniques (PCR-based syndromic panels)  
569 and standard microbiological testing on lower respiratory  
570 samples.

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

575 In statement number 12, the panel achieved high agreement  
576 (94.2%) in favor of the use of NGS on CNS biological samples  
577 when standard microbiology tests are negative in patients with  
578 suspected CNS infection (both community-acquired and  
579 healthcare-associated).

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

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

592

#### 593 **NOTE ON THE USE OF THIS CONSENSUS**

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

601 The recommendations herein are advisory, based on the best  
602 available evidence and expert consensus, and they do not  
603 establish a legal standard of care. Alternate approaches may  
604 also be valid within accepted practice standards. Ultimately, the

605 responsibility for patient outcomes lies with the treating  
606 clinicians and not with the consensus group. Importantly, close  
607 collaboration across medical specialties (i.e., a  
608 multidisciplinary approach) is essential to improve outcomes in  
609 critically ill patients, both in the initial acute phase and  
610 throughout prolonged care.

611

#### 612 **Description of the developing group**

613 The authors confirm their contributions to the paper as follows.

614 Steering Committee: VA, FC, FC, VS

615 Reviewers: SA, SB, CC, EC, IC, MC, MP, GT, MT

616 Faculty: MB, PB, EGB, GDP, FGDR, CF, GG, MG, MG, NM,

617 AO, OP, VP, FP, AR, FS, SS, CT, MT, PV, GV, MB, MF, IG,

618 LG, GG, ATP, GR, AR, MS, MS, TT, GV.

619 All authors reviewed the results and approved the final version  
620 of the manuscript.

621

#### 622 **Conflict of interest**

623 All authors declare no conflict of interest.

624

#### 625 **Acknowledgements**

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627 idea to organize this consensus. Sadly, he passed away

628 suddenly and far too soon, leaving behind the love and esteem

629 of his family and colleagues. The Authors respectfully dedicate

630 this consensus to his memory.

631

632

633

634

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Proposed Research Questions and Agreement	# Round	% Agreement
1 In critically ill patients with suspected infection, are blood cultures with fast microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	93,3
2 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, intensive care unit length of stay, hospital length of stay, 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, intensive care unit length of stay, hospital length of stay, 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 infection, is rapid microbiology testing of cerebrospinal fluid indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	86,7
6 In patients with suspected post-neurosurgical Central Nervous System infection, is rapid microbiological testing of cerebrospinal fluid indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	73,3
7 In patients with suspected abdominal infection, is rapid microbiology testing of abdominal samples indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, 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 (blood cultures 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 blood cultures with NGS microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	66,6
12 In patients with community acquired pneumonia is NGS microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit length of stay, hospital length of stay, mechanical ventilation duration?	1	43,3

13 In critically ill patients with infectious ventilator associated complications (iVAC) is NGS microbiological testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit 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 cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	40,1
15 In patients with suspected post-neurosurgical CNS infection (P) is NGS microbiological testing of cerebrospinal fluid (CSF) (I) indicated over standard microbiology tests (C) in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, 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, intensive care unit length of stay, hospital length of stay?	1	53,3
18 What are the hypothesized analytical advantages and disadvantages of Next generation sequencing (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 blood cultures with dPCR microbiology tests indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	63,3
21 In patients with community acquired pneumonia is dPCR microbiology testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit length of stay, hospital length of stay, mechanical ventilation duration?	1	46,7
22 In critically ill patients with infectious ventilator associated complications (iVAC) is dPCR microbiological testing of respiratory samples indicated over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit 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 cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, hospital length of stay?	1	63,4
24 In patients with suspected post-neurosurgical CNS infection is dPCR microbiological testing of cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, 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, intensive care unit length of stay, hospital length of stay?	1	46,7

26 Which TATs (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 pre-analytical 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

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<b>Reformulated Delphi Questions</b>	<b># Round</b>	<b>% Agreement</b>
In critically ill patients with suspected infection, is the processing of respiratory samples (BAL) with digital Polymerase Chain Reaction (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 digital Polymerase Chain Reaction (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 digital Polymerase Chain Reaction (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 (NGS) 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 CNS infection (both community-acquired and post-neurosurgical) are Next Generation Sequencing (NGS) microbiology tests of cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification rate and/or time?	2	84
In patients with suspected CNS infection (both community-acquired and post-neurosurgical) are digital Polymerase Chain Reaction (dPCR) microbiological testing of cerebrospinal fluid (CSF) indicated over standard microbiology tests in terms of pathogen identification rate and/or time?	2	80

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, ICU length of stay, and overall hospital length of stay?
5	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, intensive care unit length of stay, hospital length of stay, mechanical ventilation duration?
6	In critically ill patients with ventilator-associated pneumonia (VAP), is rapid microbiology testing of respiratory samples preferred over standard microbiology tests in terms of pathogen identification time, cure rate, mortality, intensive care unit 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 (VAP), are digital Polymerase Chain Reaction (dPCR) 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 infection, is rapid microbiology testing of cerebrospinal fluid indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, intensive care unit length of stay, hospital length of stay?
11	In patients with suspected healthcare-associated Central Nervous System infection, is rapid microbiological testing of cerebrospinal fluid indicated over standard microbiology tests in terms of pathogen identification time, pathogen identification rate, cure rate, mortality, the intensive care unit 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 (NGS) microbiology tests of cerebrospinal fluid (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 post-neurosurgical), is digital Polymerase Chain Reaction (dPCR) microbiological testing of cerebrospinal fluid (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 to not using biomarkers in terms of sepsis identification and the duration of antibiotic treatment?
15	What essential pre-analytical 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?

<b>S</b>	<b>Final statements</b>	<b>SA(7-9)</b>	<b>SD(1-3)</b>	<b>QoE</b>
#		%		
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 program 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 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 pre-analytical, analytical, and post-analytical 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 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 dPCR in terms of turnaround times (TAT) and identification rates. Furthermore, dPCR could be applied to the assessment of antimicrobial susceptibility profiles and pathogen load	96.8	0	Low

9	<p>In critically ill patients with clinical indications to advanced microbiology testing from lower respiratory tract samples, there is insufficient evidence to suggest either bronchoalveolar lavage (BAL) or bronchial aspirate (BA) for diagnosing lower respiratory tract infections when advanced microbiological techniques are applied, such as metagenomic next-generation sequencing (mNGS)</p> <p>However, in critically ill patients undergoing advanced microbiology testing on lower respiratory tract samples, the panel suggests using bronchoalveolar lavage when clinically feasible and in alignment with the logistical constraints and expertise of the operators.</p>	82.9	5.8	Low
10	<p>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</p>	87.9	0	Low
11	<p>In critically ill patients with suspected healthcare-associated central nervous system infections, the panel advises against the use of commercially available rapid microbiology testing (PCR-based syndromic panels) of cerebrospinal fluid</p>	96.9	3.1	NA
12	<p>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 (NGS) 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</p>	94.2	0	Low

13	In patients with suspected central nervous system (CNS) infections, including both community-acquired and health-care associated cases, there is insufficient evidence to suggest that digital polymerase chain reaction (dPCR) on cerebrospinal fluid offers advantages over standard microbiology tests in improving pathogen identification rates or reducing turnaround times (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, pre-analytical 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

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