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Beyond Conventional Culture: Integrating Rapid Molecular Diagnostics, Proteomic Technologies and Artificial Intelligence for Pathogen Identification in Clinical Microbiology

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ABSTRACT

This research aims to optimize in vitro propagation techniques for ginger (Zingiber officinale Rosc.) by investigating the effects of plant growth regulators (PGRs) on shoot induction, root formation, and callus proliferation. Ginger is an economically valuable crop with medicinal, culinary, and industrial applications, but its conventional propagation through rhizomes is slow, prone to disease, and limits large-scale production, necessitating improved tissue culture techniques. The highest shoot induction (4.9 shoots) occurred at 3.0 mg·L^{-1} BAP, while optimal shoot length (5.9 cm) was observed at 2.0 mg·L^{-1} BAP. Root induction was most effective at 3.0 mg·L^{-1} IAA, producing the highest root count (7.1), with the longest root length (5.3 cm) achieved at 4.0 mg·L^{-1} IAA. Callus formation was optimized using 1.0 mg·L^{-1} 2,4-D combined with 0.5 mg·L^{-1} Kinetin and 0.750 mg·L^{-1} IAA, leading to the quickest callus initiation (10 days), highest fresh weight (3.5 g), and largest diameter (2.3 cm). Acclimatization trials demonstrated high survival rates, with 93% in the growth chamber, 78% in the shade house, and 81% in open conditions. This study provides an efficient and reproducible tissue culture protocol for the mass production of disease-free, genetically uniform ginger plants, offering a sustainable alternative to traditional propagation methods and significantly improving cultivation practices, ensuring higher yields, better plant quality, and increased availability of superior planting material for agricultural and commercial applications.

Introduction

The microbiology laboratory is the source of three basic information: Do the patient have an infection, if so, what organism and what is the correct treatment? Those answers are quick and reliable, and outcomes are affected by speed and reliability (Messacar et al., 2017). The conventional microbiology takes between 48-72 hours to get a final organism identification and blood culture is less sensitive and has a longer TPD and TTS. Diagnostics are delayed and empiric and possibly inappropriate anti-bacterial treatment is prolonged (Antillon et al., 2018). This is most importantly significant in septic shock, where time to effective therapy is a key factor in survival and is the single most important factor to be delayed, resulting in progressive decline in survival. However, delayed anti-infective therapy is also linked to increased adverse events and greater costs, and sepsis is thought to be responsible for about 20% of all deaths worldwide (Dyck et al., 2024). This gap has now been reduced by new progress. Pathogens and resistance markers can now be identified from positive blood culture using commercial rapid diagnostic tests such as nucleic acid amplification tests and mass spectrometry (Ecker et al., 2010). Culture independent technologies are able to identify pathogens and resistance genes without the need for growth. Through genomic sequencing, high resolution identifies, transmission tracking and gnomically informed stewardship which will take care closer to precision medicine. Resistance gene targeting can aid quicker, evidence supported antimicrobial choices and prompt de-escalation of broad-spectrum empiric therapy when an organism is found to be resistant, otherwise delaying effective treatment and causing further resistance and complications (Peptine et al., 2026). The usefulness of these tools, for clinical use, however, relies markedly on how the results are utilized. In the meta-analysis, molecular RDT decreased time to effective therapy by over 5 hours and hospital stay by almost 2.5 days; mortality was lower with molecular RDT, but only when combined with antimicrobial stewardship program (Timbrook et al., 2016). A more recent analysis reported that rapid diagnostics and stewardship resulted in fewer deaths than traditional blood culture alone or blood culture and stewardship, and faster time to optimal therapy than blood culture alone, blood culture plus stewardship or rapid testing alone (Peri et al., 2024). The use of Mass Spectrometry for identification and stewardship oversight improved the time to optimal therapy to 23.2 hours, to effective therapy to 32 hours, and decreased the mortality rate, length of hospital stays, length of intensive care unit stays and total hospital cost from 80.9 hours, 89.7 hours, 69.7 hours, and $37,683 in costs, respectively, to antibiotic-resistant Gram-negative bacteremia cases (Kim & Yi, 2026). This evidence aligns with recommendations from the American Society for Microbiology for rolling out rapid testing of positive blood cultures, and for actively reporting the findings, reducing the time to targeted antimicrobial treatment and hospital stay for both adults and children (Wolk et al., 2025). New technologies have revolutionized the laboratory. MALDI-TOF mass spectrometry and multiplex PCR have reduced time to identification, nucleic acid amplification tests have broadened the scope of rapid molecular diagnostics, multiplexed panels and digital PCR can be used for the identification of pathogens directly from specimens without culture, and resistance determinants can be detected and multidrug resistant nosocomial organisms tracked by next-generation sequencing (NGS), which includes whole genome and targeted and shotgun metagenomics (Gwinn, MacCannell, & Armstrong, 2019; Loman et al., 2013). Artificial Intelligence has been introduced to the laboratory in recent years, for example in the area of image interpretation, spectrum analysis, sequence analysis and laboratory management (Herman et al., 2021). Despite this, traditional culture based and phenotypic testing methods are still used in parallel to these methods, and susceptibility testing is still considered as the standard method to assess resistance. The objectives of this review are to: (i) detail the advantages and disadvantages of traditional culture-based identification (ii) summaries the different rapid molecular and proteomic technologies, including their speed, accuracy, cost, and clinical applications (iii) discuss the emerging potential for AI (iv) explore the possibilities of how these methods can be integrated and validated within stewardship linked workflows and (v) outline challenges for implementation, research opportunities and future directions. Many of the primary sources cited in the figures given here are recent reviews or single center studies; in situations where, exact figures are important (e.g. turnaround times, sensitivity figures), readers are advised to check original publications and prefer established reviews.

Conventional Culture-Based Pathogen Identification

Conventional identification has been based on culture techniques which have been developed over 100 years. The process starts with the collection of a sample from the site most likely to contain the pathogen, and involves inoculation on suitable media, incubation under suitable conditions and the isolation of distinct colonies (Melrose et al., 2015). Isolation aims to obtain a pure culture, a prerequisite for reliable identification, for susceptibility testing, for studying virulence and for genome sequencing (Bertelli & Greub, 2013). This method of streaking and plating on a solid agar medium enables single colonies to be grown from a mixed sample and prevents contamination by the use of aseptic technique. Culture media are classified as liquid, semisolid or solid media and simple, enriched, selective and differential. Non-selective media are used to promote the general growth of organisms, while enriched media like blood and chocolate agar encourage the growth of fastidious organisms such as *Hemophilus influenzae (Bonnet et al., 2020*). Selective media contain inhibitory agents, like antibiotics, and/or dyes that inhibit

the growth of unwanted flora (e.g. cetrimide agar for Pseudomonas aeruginosa). Differential media incorporate indicator dyes to help differentiate organisms based on their metabolic activity, and chromogenic media use enzyme substrates such that organisms are isolated and presumptive identified in a single step (Meghana, 2020). The medium is not as important as temperature, atmosphere and incubation time: the most clinically important species are mesophilic and routine plates are usually incubated for 24 hours at 37 °C, but some organisms need special temperatures, special atmosphere, or longer periods of incubation (Fritz et al., 2012). The first morphological classification is provided by gram, acid-fast and spore stains, while the rapid, inexpensive first clue is provided by the colony morphology (size, form, margin, elevation, texture, pigment, hemolysis) (Parasuraman, Busi, & Lee, 2024). Biochemical tests (catalase, oxidase, urease, coagulase, nitrate reduction, indole, methyl red, Voges-Proskauer, sugar fermentation) provide a metabolic profile (AL-Joda & Jasim, 2021). Tube-based media (such as triple sugar iron agar) were an early development in multitest identification systems, followed by miniaturized media since the 1950s (more modern systems like the Analytical Profile Index) which reduced labour and time needed when compared to up to 20 tubes of media that would take 48-96 hours for interpretation (Altheide, 2019). Colony features, however, are highly dependent on incubation period, medium composition and under certain circumstances, the appearance of a colony may change due to stress conditions, as in the case of *Burkholderia pseudomallei* which has been subdivided into seven morphotypes (Chantratita et al., 2007). The quality of any microbiological sample is determined prior to the sample being received at the laboratory. Whether analysis is accurate depends on the specimen type, time, method, storage and transport; no downstream technology can salvage information lost due to poor collection or late transport. Aseptic collection in sterile, leakproof, labelled containers, use of aspirates, fluids and tissue where possible, collection at the optimum time (e.g. early-morning sputum for mycobacteria), and using the appropriate transport media are good practice (Pfyffer & Palicova, 2011). Recommendations for blood cultures include timing (the best time to incubation is about two hours), skin antisepsis, blood volume and sampling method. The pre-analytical phase is also the most susceptible to error in the microbiology workflow, with many steps carried out outside of the laboratory with little direct supervision. This variability is minimized with written instructions for collection sites, clear criteria for accepting and rejecting specimens, and communication between clinicians and the laboratory (Moehring et al., 2014). Direct microscopy is the oldest and quickest diagnostic test and is the first source of information that can be acted upon that is obtained, often hours or days before culture results are received. The Gram stain is the most useful: it classifies bacteria as Gram-positive and Gram-negative, it provides a clue to the bacteria's morphology, it provides an indication of specimen quality and may alter initial therapy in certain specimens including in CSF and positive blood cultures (Boyanova, 2018). It does not however, offer species level identification or resistance information. Other stains include direct examination: acid-fast stain for mycobacteria, India ink stain for Cryptococcus, Giemsa stain for blood parasites (Plasmodium), and calcofluor white stain for fungi (Makarewicz et al., 2015). The selection of stain should be based on the suspected organism; for example, fungi, Nocardia and Acanthamoeba are more readily detected by potassium hydroxide wet mount than by Gram stain in infective keratitis (Zemba et al., 2022). The advantage of microscopy is that it is fast, cheap and sometimes more sensitive than culture, but it is not as specific as culture, mass spectrometry or molecular assays, nor does it provide any resistance information. Biochemical identification has been revolutionized by automation. Instruments like VITEK 2, BD Phoenix and MicroScan are able to test many reactions at once, track growth by turbidity, colour or fluorescence, and generate patterns of identification and susceptibility from one single inoculum (Gajic et al., 2026; Salam et al., 2023). First introduced in the 1970s, automated blood culture systems are capable of continuously detecting changes in growth. Today, the whole "laboratory automation" system incorporates all the steps of specimen processing, plate inoculation, incubation and digital imaging, which allows technologists to evaluate the plates on screen with very little interference (Pence & Liesman, 2020). It has reduced turnaround time for ID and susceptibility reporting when implemented as compared to manual plating even when MALDI-TOF is already used. The automated bottle loading, subculture of positive bottles, expert-system review of susceptibility data and reporting of negative bottles reduced the overall time for a blood culture workflow from approximately 96 to approximately 61 hours, which resulted in shorter hospital stays and lower costs. But conventional identification methods still have their limitation of time, as growth is necessary for identification with automation only partially helping (Qin et al., 2025).

An isolate from culture can be directly tested for susceptibility, the information that directs stewardship. Molecular assays detect resistance genes, but resistance genes do not necessarily represent resistance phenotypes; genes may be non-functional or not expressed; resistance genes may originate from another organism in polymicrobial samples; new resistance mechanisms are not detected (Karaiskos et al., 2026). The positive culture indicates that living organisms are present, whereas the molecular methods are usually not able to differentiate if it's alive or dead or non-pathogenic organism because nucleic acid may remain. Viable isolates can also be used to perform phenotypic analysis in cystic fibrosis, such as the determination of mucoid and small colony variants (Malhotra, Hayes, & Wozniak, 2019). Pure cultures can be stored for outbreak investigation, genome sequencing, virulence studies, characterization of virulence genes, and for the study of emerging pathogens or genes for resistance. Surveillance of resistance at a national or international level relies on phenotypic characterization of isolates and the early identification of isolates is crucial in the era of whole genome sequencing used to link cases; public health laboratories are more affected by cessation of culture by clinical laboratories (Karaiskos et al., 2026). Culture is relatively-costly, standard, and easy

to transport between laboratories, and is available when sequencing or multiplex platforms are not available. Culture has not remained dormant: New media has become chromogenic, media is optimized, microaerophilic atmospheres have been developed, amoebal co-culture, shell-vial techniques, and culturomics have broadened the spectrum of organisms that can be recovered. Culture is limited in usefulness in the acute and critical-care setting in three ways (Klompas, Rhee, & Singer, 2023). Conventional methods usually take days to grow many pathogens and most are slow growing. In one prospective comparison, blood culture took around 72 hours, compared to the much quicker PCR-based detection, of up to seven days and up to 12 weeks for unambiguous diagnosis of active tuberculosis, respectively ("Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis," 2009). These delays delay targeted treatment and delay results to inform decisions at the point of impact. Low sensitivity following an antimicrobial exposure. Giving empirical antibiotics prior to sampling inhibit growth resulting in false negative cultures. For bloodstream infections, prior antimicrobial treatment decreases the sensitivity of blood culture, such as in some cohorts of sepsis, the pathogen was identified only 27% of the time, and in one study, prior therapy decreased the blood culture positivity rate by half. Sepsis is not uncommon when treating patients with clinical suspicion of sepsis, which is treated empirically without delay (Cheng et al., 2019). It is compounded by low microbial load, and suboptimal transport. Organisms that are fastidious and non-culturable. Routine media were originally designed for fast growing, undemanding organisms, like the *Enterobacterales*. Fastidious bacteria (e.g., *Bartonella, Coxiella, Rickettsia*) may require extended incubation, host cells or specialized media; auxotrophic organisms require partners to provide nutrients; dormant, persister and viable-but-non-culturable cells may not grow on a plate; and fast-growing organisms may outgrow slow-growing ones on a plate (Ughy et al., 2023). Special conditions are required for the growth of anaerobes and mycobacteria. The implications are tangible: culture-independent sequencing revealed bacteria in a markedly higher proportion of bronchoalveolar lavage samples than culture, and in a veterinary comparison culture grew 15% and 81% fewer bacteria and fungi pathogens, respectively, than did culture-independent sequencing (Vientós-Plotts et al., 2021). These are inherent limitations of the culture growth principle that won't likely be solved by marginal improvements in culture. This is because there has been a move away from culture-dependent methods that have the potential to detect fastidious, low-burden and non-viable organisms, and are sensitive in antibiotic-exposed patients. Culture also addresses a different question than molecular tests: Viability and susceptibility of a viable organism; molecular tests detect the presence of genetic material (Karaiskos et al., 2026).

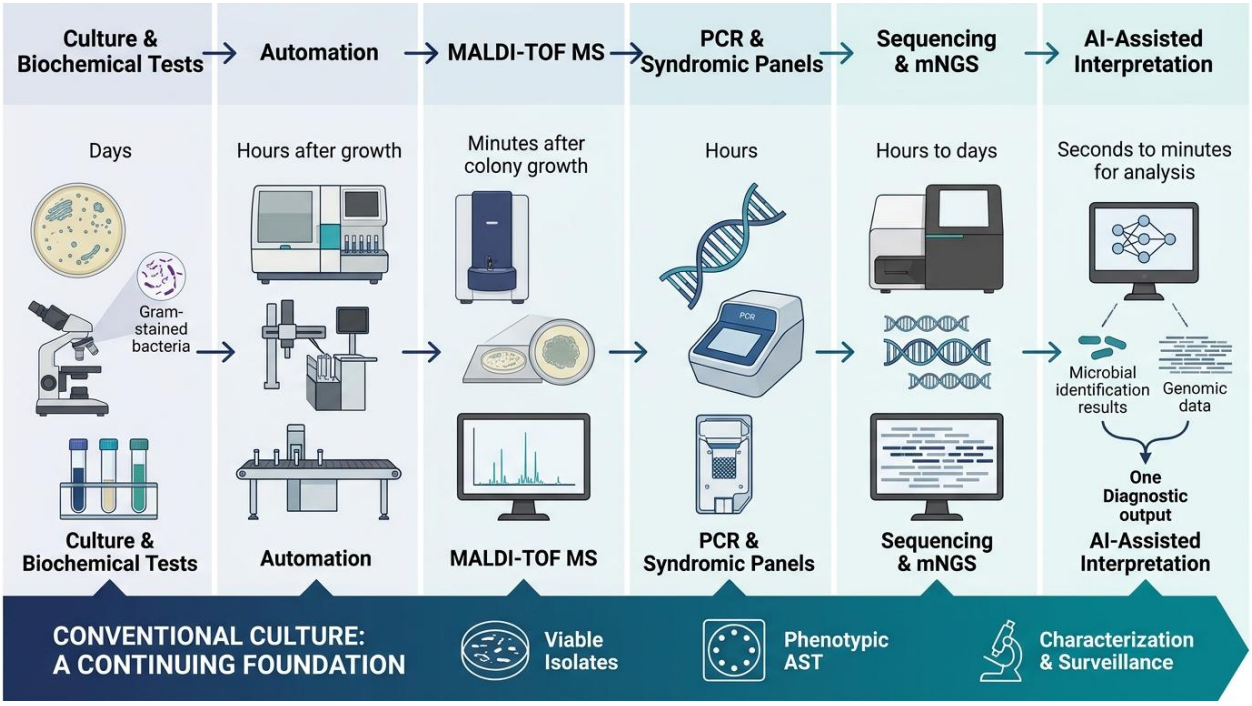


Figure 1. Evolution of pathogen identification in clinical microbiology. Conceptual timeline showing progression from culture, staining and biochemical identification (days), through automated identification and total laboratory automation, to MALDI-TOF mass spectrometry (minutes from a colony), PCR and syndromic panels (hours), and sequencing and AI-assisted interpretation, with typical time to result indicated for each stage. Culture is shown as the persistent foundation that supplies viable isolates and phenotypic susceptibility at every stage. Original diagram to be prepared by the authors; time scales are indicative and drawn from the studies discussed in the text.

Rapid Molecular Diagnostics for Pathogen Identification

Pathogen detection with molecular methods involves detecting nucleic acids, not growing organisms. In about 40 years they have evolved from being a research tool to becoming an integral part of clinical microbiology. They typically have higher sensitivity and specificity than traditional methods, shorter detection times, greater automation, and methods like quantitative PCR and NGS have higher rates of pathogen detection than culture, and often do not require cell culture (Sibley, Peirano, & Church, 2012). Almost all pathogens have either DNA or RNA genome, thus the same concept is used for bacteria, viruses, fungi and parasites. Specificity is the hybridization of target to complementary primers and probes. With PCR, a segment of DNA is copied over and over by a DNA polymerase until even small quantities of the target can be measured (Kadri, 2019). In conventional PCR the result is only observed at the end of the PCR reaction, usually after gel electrophoresis, fragment analysis or sequence. Due to this similarity in the amount of product generated in the end of the cycles, the end-point measurement is qualitative or at best semi-quantitative (can detect about a 10-fold change) and opening the tube after amplification increases the chance of contamination (Itahashi et al., 2010). Real-time (quantitative) PCR is a technique that integrates amplification and detection in the same tube and in real time. An optical system is incorporated in a thermocycler for monitoring the fluorescence level and the cycle at which the exponential amplification is first detectable is related to the number of initial copies and can quantify and detect a twofold change (Higuchi et al., 1993). Fluorescence can be due to the intercalating dyes, e.g. SYBR Green which are inexpensive but not sequence specific, or to probe chemistries (hydrolysis probes, molecular beacons, Scorpion probes) which provide additional sequence specificity. With the addition of reverse-transcriptase qPCR, quantification can now be applied to RNA targets (Steward, 2022). Closed-tube: is faster, avoids post-PCR manipulation, reduces contamination and increases the reproducibility, but costly. Conventional PCR is thus still used where presence/absence is needed or resources are limited while qPCR is used where speed, quantification and contamination control are important (Harteveld, Kleanthous, & Traeger-Synodinos, 2009). Nucleic acid amplification tests are especially valuable for screening colonization (MRSA, VRE) as they detect the pathogen and resistance determinants much more quickly than culture. Multiplex amplification of a clinical syndrome, not a single suspected organism, is performed by syndromic panels (Wang & Chen, 2026). By definition of the experts, a panel identifies 3 or more pathogens and genotypic resistance markers (where applicable) in less than 6 hours. Commercial panels are usually comprised of real-time PCR, automated sample-to-answer platforms (such as the BioFire FilmArray and Unyvero) and include bloodstream infection, pneumonia, meningitis and encephalitis, gastrointestinal and respiratory infection (Heo & Kim, 2026). Panels typically yield better sensitivity and diagnosis around significantly shorter turnaround times than conventional techniques. For acute gastroenteritis, time from collection to results decreased from 47 hours (culture) to 18 hours and time from collection to antimicrobial therapy decreased from 72 hours to 22 hours (Jiménez-Jiménez et al., 2025). Among children who were suspected of having a central nervous system infection, a 14-pathogen meningitis and encephalitis panel shortened the duration of antimicrobial therapy, length of stay and overall hospitalization costs. A rapid multiplex respiratory panel was more diagnostic than immunofluorescence (81% vs. 31%) and results were obtained in less time (less than 2 hours vs. 26 hours) and led to withholding antibiotic treatment in adults and children in an emergency department study (Echavarría et al., 2018). In one study, rapid RT-PCR influenza testing, which yielded results in less than 30 minutes, changed management in 61% of cases, and the cost savings more than covered the cost of the tests. The panels are believed to have a beneficial effect in improving the accuracy of diagnosis and to assist in stewardship for critically ill patients, but without a proven mortality benefit from trial data. Limitations are real. Panels are costlier than single plex tests (but can be offset by reduced overall care costs), some of the targets are not necessarily clinically relevant as nucleic acid detection does not rule out or rule in disease, limits of detection are typically higher than optimized in-house real-time PCR, some microbes are not included in the panel and reimbursement barriers may limit access to the test. Therefore, diagnostic stewardship is necessary: diagnostic panels are most helpful when ordered for syndromes and patients in whom the results will alter management and when the results are shared with the treating team. Isothermal methods amplify nucleic acid at constant temperature, eliminating the need of thermocycler. They include loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), nucleic acid sequence-based amplification (NASBA), strand displacement, rolling circle and helicase-dependent amplification. They have shorter reaction times and simpler instruments than PCR, and tolerate inhibitors better than PCR; some meet the WHO ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, deliverable) for point-of-care tests (Neumann, Madaboosi, & Nilsson, 2022). Developed in 2000, LAMP uses four to six primers that recognize six regions of the target sequence and a strand-displacing polymerase (usually Bst) to amplify the target sequence at 60-65 °C, typically without specialized equipment, and with a comparable time of amplification (about 30-65 minutes) to PCR (Notomi et al., 2000). The major limitation is that it is complex to design the primers. RPA is species-independent and uses a recombinase-primer complex to invade double-stranded template at 37-42 °C to produce millions of copies in a single tube in ~ 40 minutes, with just a single primer. The combination of LAMP or RPA to CRISPR-Cas system provides an additional layer of sequence recognition and lateral-flow readout, while digital formats allow to quantify the results absolutely. Several new point of care technologies can provide results in about 6-15 minutes, but have not yet been tested in the field. As with all amplification assays, isothermal assays identify nucleic acids, not living organisms and do not

provide phenotypic susceptibility, and should be validated against accepted assays prior to clinical use. Targeted NGS is in between syndromic PCR and unbiased metagenomics. It adds a set of targeted pathogen and resistance genes to a short list of targets prior to sequencing, either by multiplex PCR amplicons or by hybrid capture (CRISPR-based enrichment has also been described). Relative to metagenomic and whole genome methods, sequencing can be concentrated on targets, resulting in lower numbers of hosts and higher sensitivity in lower burden samples and mixed infections, at relatively low cost. About 13 hours was needed for detection of tNGS, approximately 17 hours required for metagenomic sequencing, and approximately 61 hours was required for culture in the urinary tract infection multicentre comparison, with polymicrobial infection present in most samples (Chang et al., 2025). A nanopore targeted assay was comparable to metagenomics in terms of positivity rate (around 70%) and performed better than blood culture with results available in approximately 7 hours for the majority of samples in bloodstream infection. Culture-negative and polymicrobial PJI's were identified by targeted 16S sequencing. The compromise here is 'breadth': tNGS cannot detect unexpected or novel organisms, and resistance detection is limited to known genes. Metagenomic NGS (mNGS) is the most flexible method. All nucleic acids are sequenced in a specimen and the sequence is compared with reference DNA to detect organisms, including rare or novel pathogens, and at the same time to look for resistance genes and virulence factors – in one hypothesis-free, culture-independent assay. The workflow consists of nucleic acid extraction and library preparation, high-throughput sequencing and bioinformatic taxonomic assignment. One study was able to identify pathogens within a median of 50 minutes of sequencing and approximately six hours after sample receipt using the cell-free DNA from infected body fluids and nanopore sequencing, and the assay was positive in 58% of samples that were culture- and PCR-negative but eventually confirmed infectious (Blauwkamp et al., 2020). Pooled analysis gave a median of 29 h from collection to result (range 4-144 h) which depends greatly on workflow and setting. Diagnostic performance is fair but variable with the specimen. In 511 samples, mNGS detected more isolates than culture (50.7% vs. 35.2%) and was less inhibited by prior antibiotic use, being more sensitive than culture for M. tuberculosis, viruses, anaerobes and fungi. Meta-analysis of 21 studies (2023 samples) indicated that pooled sensitivity and specificity for blood samples were 90 and 86, respectively, for CSF, 75 and 96, respectively, and for orthopaedic samples, 84 and 67, respectively (Govender et al., 2021). For bacteria, 79% of the body fluids were sensitive and 91% of the body fluids were specific in Illumina and 75% and 81% in nanopore (Blauwkamp et al., 2020). For VAP, the nanopore assay had a median turnaround time of 4.4 hours compared to 72 hours of culture for the gold standard, and showed 91.3% sensitivity and 78.3% specificity (Huang et al., 2023). mNGS has also identified new organisms not detected by other tests in immunocompromised patients and in mixed infections, although conventional tests are not a good reference standard in these settings and large differences in sensitivity have been reported in individual studies and should be interpreted in the context of these patients. Respiratory mNGS has the highest sensitivity in bronchoalveolar lavage fluid and is reported to be approximately 73% in lavage fluid compared to 35-40% for blood, CSF and pleural fluid – in one single centre report. A positive rate of 60.6% was achieved for mNGS compared to 20.2% for routine CSF examinations in CNS infection (Zhang et al., 2023). 16S rRNA and fungal ITS sequencing is a more cost-effective targeted method, and in one study, 91.8% of the culture-positive samples were concordant with the sequencing results (Abayasekara et al., 2017). One of the most clinically valuable skills of molecular techniques is the ability to detect resistance at the genetic level. Multiplex PCR, microarrays, whole-genome and metagenomic sequencing, and new, emerging technologies such as CRISPR can identify resistance genes in just hours, even from polymicrobial samples not purified. Commercial panels have resistance markers built in (the pneumonia panel contains seven and the blood culture identification panel ten genes). Six bacterial pathogens and fourteen resistance genes were identified with high concordance to the observed phenotype using a multiplex real-time PCR for lower respiratory infection in single infection. When identified, susceptibility testing requires an extra day or two; sequencing-based prediction can substitute for this extra time. The machine-learning models achieved approximately 95-100% accuracy for several antibiotics in K. pneumoniae and 96.6-98.4% in A. baumannii, and the predictions matched phenotyped results in 50 prospective samples and were 100% accurate in the same samples for four of the five antibiotics; the models predicted resistance results within about 19 hours compared with 63 hours for culture-based results. Probe-based enrichment of resistance genes (capture mNGS) had a median time to result of approximately 25 hours versus 73 hours for standard metagenomics, and was 44-fold more sensitive than standard metagenomics, with blaCTX-M showing 100% sensitivity and specificity for ceftriaxone resistance, and blaKPC showing 94% sensitivity and 100% specificity for carbapenem resistance (Zhou et al., 2026). The picture across studies is not as clear. A nanopore-versus-Illumina comparison showed that sensitivity was approximately 77%, specificity approximately 83% and accuracy was 80% against phenotype; and a meta-analysis showed that there was 88% categorical agreement and very major and major error rates of 24% and 5% respectively, with a significant proportion of truly resistant organisms reported as susceptible. Genotypic assays only detect markers for which they have a probe, results can be overcalled in presence of the gene which is not functional or not expressed or from a non-pathogen, the absence of a gene doesn't mean susceptibility. Phenotypic testing is therefore the reference test and genotypic is used as a guide for early therapy. The only limitation of sequencing is its capability of detecting all nucleic acid—whether alive or dead, from any origin or clinical relevance. Contamination. False-positive microbes are from environmental contamination, reagent contamination, kit contamination and cross-sample contamination (including index misassignment), with approximately two-thirds introduced in the wet lab in an external QA of lower respiratory infection testing (Miller et al., 2025). The pillars of

mitigation are extracting blanks, using negative controls, batch modelling and contamination-aware filtering. In low biomass samples the contamination may be too much of the signal. Host background. In many specimens, human DNA precludes analytical sensitivity and leads to false negatives, especially in samples with low biomass, such as Cryptococcus and M. tuberculosis which are hard to lyse. Negative test results do not rule out infection. Interpretation. In one evaluation, non-viable organisms, normal flora and clinically insignificant agents (e.g., anelloviruses) were mistaken for infectious agents, misidentification of the target species in mixtures is challenging, and 11% to 39% of laboratories were not able to arrive at an etiological diagnosis even when the target had been detected. Unexpectedly discovered abnormalities may result in unwanted therapy. Standardization. The pipelines vary in how they are annotated for resistance genes, the pipelines lack end-to-end validation, incidental human genetic discoveries pose ethical dilemmas, and the cost and regulatory requirements reduce adoption. These factors should be considered when considering the use of mNGS selectively (immunocompromised patients or critically ill patients, unexplained cases and when the results of the initial tests are negative), and when interpreting the results, always in conjunction with the clinician and microbiologist. The strongest applications are in lower respiratory infection (best with lavage fluid, including difficult organisms such as nontuberculous mycobacteria, Chlamydia and parasites, and invasive pulmonary fungal infection where fungal read abundance and cut-offs may support diagnosis), bloodstream infection and sepsis (where blood culture sensitivity is poor, especially after antibiotics; one study reported that combining blood culture with mNGS significantly reduced 28-day mortality, a finding needing confirmation in larger controlled studies), and meningitis and encephalitis of unknown cause (where a positive CSF result is strongly informative but a negative one does not rule out infection, and detection of fungi in normally sterile CSF can change therapy). Orthopaedic specimens show lower specificity (67%), reflecting difficulty distinguishing infection from colonization (Govender et al., 2021). Specimen choice matters as much as the platform: even unbiased methods depend on sample condition, and bone and marrow samples in one formalin-fixed tissue study often failed because of nucleic acid degradation.

Table 1. Molecular and proteomic diagnostic platforms for pathogen identification. Values are indicative and drawn from the studies discussed in the text; they are not clinically validated protocols.

Platform	Principle and target	Typical specimen	Time to result	Key strengths	Major limitations
Culture and biochemical identification	Growth, colony morphology, enzymatic profile	Any; isolate required	48-72 h to identification (up to days or weeks for fastidious organisms)	Viable isolate, phenotypic susceptibility, low cost, surveillance	Slow; reduced yield after antibiotics; fastidious organisms missed
Conventional PCR	Gel- or sequence-read amplicon of defined target	Isolate, clinical specimen	Hours	Simple for presence/absence	Qualitative; contamination risk from open tubes
Real-time (quantitative) PCR	Closed-tube fluorescence detection of defined targets	Clinical specimen, positive blood culture	About 1-3 h	Quantitative, closed-tube, sensitive	Cost; fixed targets; nucleic acid does not prove viability
Syndromic multiplex panels	Multiplex PCR, sample-to-answer	Blood culture, CSF, respiratory, stool	Under 6 h (about 1-3 h typical)	Broad differential, resistance markers, stewardship benefit	Cost; limited panel; uncertain clinical relevance of some targets
Isothermal amplification (LAMP, RPA, CRISPR-coupled)	Constant-temperature amplification	Clinical specimen, point of care	About 15-60 min	Simple instruments, portable, inhibitor-tolerant	Primer design (LAMP); limited multiplexing; needs field validation
Targeted NGS	Enriched predefined pathogen/resistance targets	Blood, CSF, urine, joint, respiratory	About 7-24 h	Sensitive for known targets, mixed infections, lower cost	Misses unexpected organisms; resistance limited to targeted genes

Metagenomic NGS	Untargeted sequencing of all nucleic acid	Lavage, CSF, plasma, tissue	About 6-30 h (median 29 h pooled)	Hypothesis-free; rare and novel pathogens; resistance genes	Host background, contamination, interpretation, cost, standardization
MALDI-TOF MS (isolate)	Ribosomal protein spectrum matched to a library	Colony; positive blood culture pellet	Minutes (about 20-60 min from broth)	Rapid, low cost per test, high accuracy	Needs growth; library-dependent; limited resistance information
LC-MS/MS proteotyping	Peptide sequences matched to genome-derived databases	Isolate; complex samples (research)	Hours	Species/strain resolution, virulence and resistance proteins	Complex instruments, bioinformatics; mainly research use

Proteomic Technologies in Clinical Microbiology

No single technology has revolutionized routine identification like mass spectrometry. To identify an organism, mostly highly expressed ribosomal proteins are subjected to protein analysis by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. The sample is co-crystallized with a matrix, and the ions are ionized by the laser, with the time of flight of the ions measured to produce a spectrum, which is checked against a reference library to determine the genus, species and sometimes subspecies ("MALDI-TOF MS: A reliable tool in the real life of the clinical microbiology laboratory," 2024). Preparations are easy and identification does not take more than minutes after loading the target plate. Bacteria, yeasts and filamentous fungi are tested with both types of instruments which are used widely, and, to a lesser extent, parasites and viruses. Today it is considered the standard of the day for rapid microbial identification in many hospitals. The main advantage is the amount of time. About a day's worth of lab work is saved with identification now taking less than an hour after the culture has been completed, as opposed to 24-48 hours via biochemical testing. Correct species-level identification of routine isolates was obtained in approximately 84-94% of instances (van Veen et al., 2010); MALDI-TOF was as efficient as VITEK 2 (species-level error rates of 5.56 per cent compared with 6.24 per cent) and was able to identify yeasts rapidly and accurately against API ID 32C (Chen et al., 2014). Made more species-level identifications than traditional workflow (including manual biochemical testing, latex agglutination, fatty acid analysis and 16S/ITS sequencing for hard-to-identify organisms), required fewer reagents and shortened labour and turnaround by 2-3 days in a hospital laboratory. For Brucella, all strains were correctly identified to species level by MALDI Biotyper while an automated biochemical system correctly identified 88-96% ("An evolution of brucellosis infection control," 2022). It has been suggested to use MALDI-TOF routinely and only to use biochemical identification as a second test if MALDI-TOF cannot identify it with confidence, thereby lowering the turn-around and marginal costs while keeping the accuracy of the results. In addition, MALDI-TOF is faster, less expensive and more specific than traditional sequencing, which is used only when the organism cannot be identified using other methods. The economics are odd, because the per-sample costs are quite low, typically about USD 0.50 for some applications, and the capital cost is high, so the savings in reagent and labour can offset the capital cost within a few years. This has resulted in widespread adoption but instrument costs are still high in many low-income situations. Applications are now a part of strain typing and epidemiology, toxin or virulence factor detection, and resistance mechanism studies. Culture independent methods have also been developed for blood, urine and faeces, but the reliability of these depends on factors like the number of bacteria present. Positive blood culture. The conventional method involves subculture (overnight 18-24 hours). Purification of a bacterial pellet from a positive broth can yield a result in less than an hour; the result of one in-house method required 20 minutes, and was 88.6% accurate as against subculture (93.3% for Gram-negative and 81.6% for Gram-positive bacteria, excellent for anaerobes and 20% for yeasts) (Stevenson et al., 2010). This still requires culture growth, and does not make culture independent. Urine. With few bacteria and little host protein, urine is the specimen most often proposed for direct analysis. Direct MALDI-TOF/TOF was reliable for genus level identification of monobacterial samples, but mixed infections are not so easy ("Identification of pathogens from native urine samples by MALDI-TOF/TOF tandem mass spectrometry," 2020). Blood and CSF with low pathogen load usually require culture enrichment to achieve detection levels ("MALDI-TOF MS: A reliable tool in the real life of the clinical microbiology laboratory," 2024). Resistance detection. There are three approaches: detection of the enzymatic activity, such as β-lactamase assays, in which the mass peaks of an antibiotic shift after incubation (in a few hours); comparison of the spectra of bacteria grown with and without antibiotic (including stable-isotope labelling); and detection of the peaks of the resistance biomarker or the protein fingerprint of resistant clones. Mass-spectrometric techniques can provide time of resistance in hours instead of 24-72 hours for culture-based susceptibility testing. Most are based on a cultured isolate, designed

for specific drug/organism combinations and only recognise patterns with which they were trained; evidence is primarily from single centre evaluations. Conventional susceptibility testing is the reference and results should be used to inform early treatment. Only adequate reference spectra can be used for identification, so rare or poorly represented species may be misidentified or not identified, closely related species have similar spectra and are difficult to separate, polymicrobial cultures yield mixed spectra that are difficult to interpret, direct tests of low-load specimens are restricted by minimum bacterial numbers, and spectral interference from endospores has been reported ("MALDI-TOF MS: A reliable tool in the real life of the clinical microbiology laboratory," 2024). For dermatophytes there is no meaningful time saving, and the same applies for the use of the method for parasites and viruses. Growth is the rate limiting step in most normal workflows, still starting from a colony or a positive culture. Does not replace susceptibility testing, culture independent detection and sequencing for what it cannot resolve. The second important soft ionization method is called electrospray ionization (ESI). The liquid normally comes from liquid chromatography (LC) is sprayed through a charged needle to produce multiply charged ions of proteins and peptides that couple naturally to tandem MS and are the basis of most proteomic studies of microorganisms. In bottom-up (shotgun) proteomics, peptides are produced by digesting proteins (usually with trypsin) and are separated by liquid chromatography and analysed by tandem mass spectrometry (LC-MS/MS), which can be used to identify proteins of any organism with a sequenced genome or to characterize virulence and resistance determinants as well as identity. Top-down proteomics identifies intact proteins at high resolution, introduces no information loss due to digestion and can identify proteoforms: one optimized platform was able to distinguish between near-identical pathogenic species which are poorly discriminated by MALDI-TOF. These techniques are quantitative like iTRAQ and label-free quantification that compare the resistant versus susceptible strains or bacterial reactions to a drug. Another application of ESI is in conjunction with nucleic acid amplification (PCR-ESI-MS) in which a broad-range PCR amplicon is measured using time-of-flight mass spectrometry with the organism inferred from base composition in the case of bacteria, fungi, viruses and resistance genes ("Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacterial and Candida bloodstream pathogens," 2010). The information presented shows that an unbiased proteomics workflow was able to identify both species and resistance related proteins within less than 4 hours from a primary culture, using 98% sensitivity and 100% specificity for the seven species and eleven resistance determinants (Blumenscheit et al., 2025). Ambient ionization methods (DESI, DART) and handheld probes can analyse samples with little or no preparation, with a handheld probe classifying bacteria by Gram stain, genus and species within seconds in an early-stage study. These approaches add depth, resolution and the ability to analyses mixtures, but need complex instrumentation, longer workflows, bioinformatics expertise and standardized databases. They are mainly research and reference-laboratory tools that extend, rather than replace, MALDI-TOF. Proteomics also contributes to host-response diagnostics and multi-omics integration (Section 7). No single method is best everywhere. Traditional biochemical testing generally takes 2-5 days after isolation, is complex and somewhat subjective, but is simple, inexpensive and still relevant as a second-line method. MALDI-TOF takes minutes from a pure colony; targeted molecular identification can be completed in under an hour; and PCR- and CRISPR-based assays offer higher sensitivity and the ability to identify genetic resistance mechanisms. Sequencing methods add resolution and breadth, but their cost, equipment and expertise requirements restrict routine use. Conventional susceptibility tests are inexpensive and widely used but slow and labor-intensive and cannot assess non-cultivable bacteria, whereas molecular approaches are faster but detect only genetic determinants.

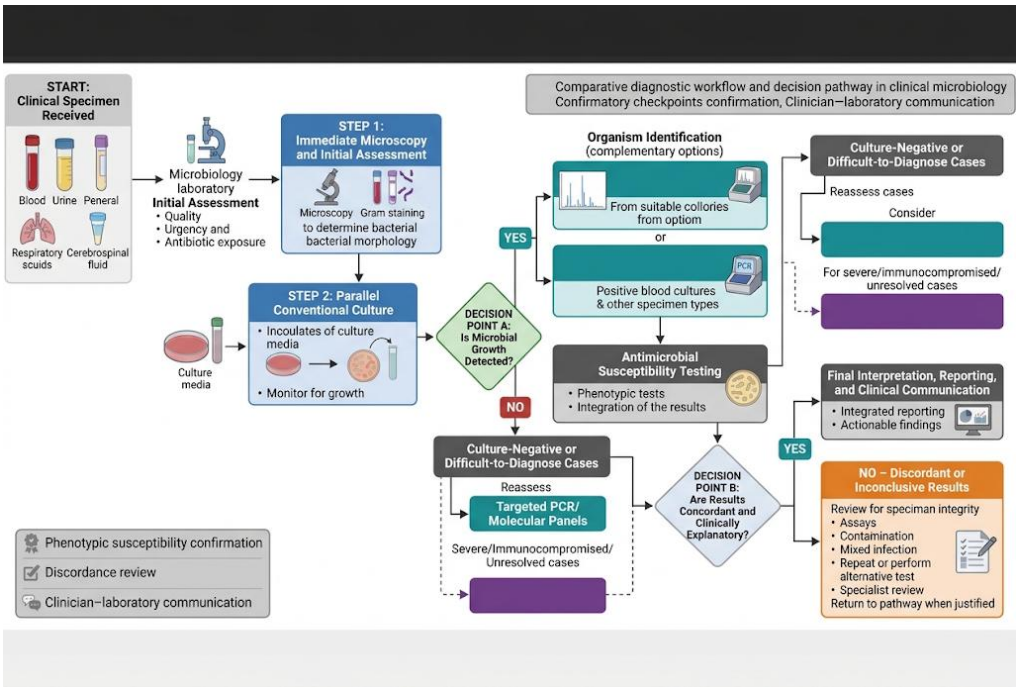


Figure 2. Comparative diagnostic workflow and decision pathway. Flow diagram showing how specimen type, clinical urgency and initial results guide test selection: direct microscopy and Gram stain on receipt; parallel culture; rapid identification from a colony or positive blood culture by MALDI-TOF or a PCR panel; syndromic or targeted molecular testing for culture-negative or antibiotic-exposed patients; and metagenomic sequencing reserved for unexplained, immunocompromised or severe cases after first-line tests fail. Confirmatory checkpoints (phenotypic susceptibility, discordance review, clinician-laboratory communication) are marked. Original schematic to be prepared by the authors; it illustrates a conceptual workflow and is not a clinically validated protocol.

Artificial Intelligence in Diagnostic Microbiology

Artificial Intelligence is a vast area of computer science which involves creating systems to carry out tasks traditionally associated with human mental ability. Machine learning (ML) refers to a type of learning in which a system learns relationships from data without being explicitly programmed, and deep learning (DL) is a subfield of ML that involves learning representations directly from raw data with multilayered neural networks. Supervised learning: Each training example is labelled, with the model being taught to predict the label, whether performed by logistic regression, support vector machines, random forests or gradient boosting; unsupervised learning: Structure is found in the unlabeled data (for example clustering of isolates); semi-supervised learning: A small number of labelled data is used alongside a much larger number of unlabeled data. Convolutional neural networks (CNNs) are the preferred choice for image analysis, recurrent networks and transformers for sequences; records are being summarized by large language models and reports interpreted. The training, tuning and test sets are used to prevent overfitting and sensitivity, specificity, precision, F1 score and area under the ROC curve are the criteria used to measure performance. Classical ML is enough for structured data, easier to interpret, while deep learning requires bigger datasets, but is opaquer and can achieve higher performance on images and sequences. There are three main types of data produced by microbiology, with each type dealt with by a different type of model, image data from stained smears or culture plates, mass spectra, sequence data. In all three, models are playing where the volume or subtlety of the data is too large to be reviewed by a technologist. Gram stain interpretation. The Gram stain of a positive blood culture is sometimes the first laboratory result that a clinician sees but it is subjective and requires experience to be interpreted and may not be available during the night. Automated microscopy can digitize slides and CNNs can be trained on digitized blood culture smears that are positive for the presence of bacteria, where the morphology of the bacteria (cocci in clusters or chains, rods) can be classified on screen. The use of vision transformers and digital holographic microscopy, along with AI is proposed, and similar methods are used for detection of rare acid-fast bacilli. There are still finer distinctions to be made, mixed populations are still problematic, and atypical staining and artefacts (stain precipitate, debris) are still hard. Public annotated datasets are small and represent specific stains and microscopes. Culture plate reading and colony counting process. The interpretation of plates is subjective and repetitive. Adaptive thresholding and watershed segmentation were combined with classical image-processing tools, yielding counting errors of approximately 4.9% and 3.8%, respectively, on the public AGAR dataset, and a YOLOv8-based detector reported counting error > 93% and real-time speed. Imaging systems can provide quantitative information about the morphology and colour of the colonies, and are said to be better able to detect small and mixed colonies than the human eye.

Urine plates are already classified as commercial products by colony count, and are already sorted into no growth (which can be reported without a technologist) and growth requiring review, with FDA clearance for the latter already for the first one. The results from these are predominantly based on research data and require verification locally; appearance of colonies is not enough to identify an organism. In total laboratory automation packages, AI-powered culture reading can autonomously release negative cultures, decreasing unnecessary isolation and antibiotic therapy, and robotic platforms have been said to take care of up to 70% of samples. Conventional identification involves matching a spectrum to a library, which in most cases is effective for common organisms, but in some cases, it doesn't get coverage for uncommon organisms and is not effective for closely related organisms. ML considers the entire spectrum as a high dimensional pattern. Reported improvements are a model based on XGBoost which was able to classify eight Salmonella serotypes with AUC around 0.97-0.98 (“Automated identification of Salmonella serotype using MALDI-TOF mass spectrometry,” 2025), a random forest model that achieved an accuracy of 0.991 for Enterococcus species while the commercial database could only reach an accuracy of 76.9% and a pipeline of support vector machine models that reached an F1 score of 97% for Mycobacterium abscessus subspecies. These studies were often not clinical and were small in size, demonstrating feasibility, not clinical performance. A benchmarking study of nearly 100,000 spectra, from over 1,000 species, revealed that the most difficult challenge is to identify new species, and that neural networks with Monte Carlo dropout can classify an unknown spectrum as "other" but not to mistakenly classify it as a known species (“Bacterial species identification using MALDI-TOF mass spectrometry and machine learning techniques,” 2021). The best developed application is for predicting resistance from routine spectra without further incubation. A large study used a public database of more than 300,000 spectra and over 750,000 resistance phenotypes from four institutions to train calibrated models, with best results obtained with gradient-boosting methods like CatBoost (Weis et al., 2022); models for S. aureus, E. coli and K. pneumoniae have been created, and resistance to carbapenems in K. pneumoniae has been classified using random forest, SVM, logistic regression and XGBoost. Transfer of models between instruments, however, was not very successful and peaks could be due to lineage as well as the resistance mechanism, therefore local validation is required. The problem is that Spectra are noisy and high dimensional and have many more peaks than isolates so this is corrected by using autoencoders, or feature selection (Boruta), or semi-supervised self-training and batch correction (ComBat). In most sequencing workflows, AI plays a role in every step, including quality control, variant calling, pathogen classification, genotyping, tracking and recognizing new pathogens in metagenomic sequencing, and the central issue of untargeted sequencing—what is important of many detected organisms? Large collections of genome are predicted for resistance using machine learning based on gene content, with single species models being the most accurate as summarized in Section 3.6. Large language models have been suggested to enable access to bioinformatic analysis for non-specialists, but output from these LLM's needs to be carefully checked. AI variant prioritisation is more developed in human genomics and provides insights into the potential applications pathogen genomics could use. As well as relying on models, AI integration also requires information infrastructure. The laboratory information system (LIS) should provide accurate, real-time access to data and poor interoperability and data structure are significant barriers. AI can detect duplicated test orders, aid automated verification of results, facilitate smart reflex testing, and guide de-escalation or flag a resistance marker at point of result, bringing rapid diagnostics to stewardship where there is integration. Results should be accompanied by metadata (specimen type, prior antibiotic exposure, transport time, instrument, reagent lot, database version) so that models and interpreters can judge them. AI is also used for documentation, audit trails and quality management. A model that performs well where it was developed may fail elsewhere, and much published work has been hard to replicate, usually because of data leakage (for example, the same patient or isolate in training and test sets), lack of standardization and inadequate methodological expertise. Validation should proceed through internal validation (cross-validation), external validation on independent data, and prospective validation, with performance reported using discrimination, calibration and error profiles rather than accuracy alone. Bias may arise from narrow cohorts (isolates from a single hospital), imperfect labels (a susceptibility method that itself has error), algorithm design and mismatches between development and deployment settings. Explainability supports trust: showing which spectral peaks drive a resistance call lets a microbiologist judge biological plausibility, though explanations must themselves be validated to avoid automation bias. Reporting should follow recognized checklists (for example, the EFLM checklist for AI and ML studies based on laboratory data) and data should follow FAIR principles. Models should be verified on local data and monitored after deployment because performance drifts as instruments, reagents, populations and resistance patterns change. A trained professional remains accountable for the final result. Regulation and data protection shape adoption: software influencing diagnosis or treatment is generally regulated as a medical device; in Europe the In Vitro Diagnostic Regulation, ISO 15189:2022 and the AI Act together define responsibilities, and in the United States CLIA-based models have been proposed as a template for clinical AI accountability. Health data are protected by laws such as HIPAA and GDPR, and metagenomic data contain human DNA that can reveal incidental genetic findings. Integration with hospital systems raises cybersecurity risks, and questions of bias, transparency, deskilling and medical liability remain. Requirements differ by jurisdiction and are changing, so laboratories must confirm which apply before deployment.

Table 2. Artificial intelligence applications in clinical microbiology laboratories. Summary of reported applications; performance figures come from research studies and need local validation.

Task	Input data	Typical models	Potential benefit	Validation needs and limitations
Gram stain interpretation	Digitized smear images	CNNs, vision transformers	Faster, more consistent first-line reading; out-of-hours support	Small, single-site datasets; artefacts and mixed populations; validation across stains and scanners
Culture plate reading and colony counting	Plate photographs	Faster/Cascade R-CNN, YOLO, classical segmentation	Automated no-growth release, colony counts, early growth detection	Medium- and camera-specific; overlap, haemolysis, pigment; cannot identify species alone
Species identification from MALDI-TOF spectra	Mass spectra	Random forest, SVM, XGBoost, autoencoders	Resolution of closely related taxa; flagging unknown species	Mostly non-clinical or small collections; instrument and site transfer
Resistance prediction from spectra	Routine MALDI-TOF spectra	Gradient boosting (CatBoost), neural networks	Susceptibility call with no extra incubation	Lineage confounding; poor cross-instrument transfer; local validation required
Resistance and pathogen prediction from sequences	Reads, assemblies, gene content	Single-species ML models, deep learning	Hours-to-days saving over phenotypic testing; novel pathogen recognition	Organism- and antibiotic-specific accuracy; very major errors; database dependence
Laboratory decision support and management	LIS and EHR structured data	Supervised ML, rule/ML hybrids	Test utilization, result verification, stewardship alerts, audit trails	Interoperability, alert fatigue, governance, drift monitoring

Comparative Evaluation of Diagnostic Technologies

Comparative evaluation compares alternative assays, platforms or algorithms against a reference assay or platform, typically in terms of the sensitivity, specificity, speed and practicality of each. In different contexts the terms have different meanings. Rapid tests performed better than enzyme immunoassays for hepatitis B surface antigen (HBsAg) with pooled sensitivity of 90.0% and pooled specificity of 99.5%, but were less analytical sensitive (any test that could detect less than 50-100 times less than EIA), and had higher limits of detection ("Diagnostic accuracy of tests to detect hepatitis B surface antigen," 2017). Sensitivity and specificity also depend on the context, and the estimates are biased due to an imperfect reference standard. This is important in a broad range of contexts, such as culture, where only mixed infections are cultured; and discrepant analysis, in which only discordant samples are recultured, which is statistically biased in favour of a more sensitive test. Just because it is accurate, does not make it valuable. A high diagnostic accuracy test can make little difference to patient outcomes, and is therefore dependent on clinical evidence, which is a mix of scientific validity, analytical performance and clinical performance. Analytical validity, clinical validity, clinical utility and regulatory alignment, and randomized trials to evaluate the link between test results and therapeutic decisions are ways to measure meaningful outcomes in the context of a framework. Weaker designs tend to overestimate performance: case-control designs are vulnerable to spectrum bias; and a meta-epidemiological study identified that differential verification and case-control validations led to significantly higher diagnostic odds ratios (DORs) than cohort designs. The turnaround time (TAT) is related to the accuracy of the test, but the definitions of TAT differ (TAT is defined as receipt-to-report time inside the laboratory or as request-to-decision time). Randomized trials of POC tests have reported median TATs of 14 minutes compared with 55 minutes in the central laboratory and reductions of 56.2 minutes in the TAT in the laboratory and 75.2 minutes in time to clinical decision in ED in a recent multicentre trial ("Point-of-care testing improves care timeliness in the emergency department," 2024). Molecular influenza testing at the bedside, with a TAT as short as 20 minutes, decreased the length of time spent in the ED by an average of 33 minutes, and very rapid TATs were linked to earlier discharge and earlier discontinuation of antibiotic use, indicating potentially a narrow therapeutic window where results could influence management; in centralized laboratories, TATs ≤ 2 hours are unlikely. In addition to the analytical step, speed loss can also occur at other steps in the workflow, e.g., printing, sorting and dispatching, in one Kenyan tertiary hospital, speed loss was observed at these post-analytical steps and delays reached up to 8 hours. Multiplex panels and sequencing expand the range of pathogens that can be covered from a single specimen. In one study of mixed pulmonary infection, mNGS showed a high sensitivity compared with conventional tests (97.2%), with 18 more pathogens detected in patients with primary

immunodeficiency (“Metagenomic next-generation sequencing for mixed pulmonary infection diagnosis,” 2019; “Clinical application of metagenomic next-generation sequencing for suspected infections in patients with primary immunodeficiency disease,” 2021), and in another study, 51% of 169 mixed infections were bacterial-viral. The uncertainty of the conventional reference must be considered in interpreting such large gaps. Horseshoe collection methods are constrained by specimen choice including the use of collection time window that spans 1-3 days after onset of symptoms, the use of appropriate collection devices and pathogen-specific guidelines for collection. Cost is very dependent on what is counted. Reagent-only costs are low and declining (currently USD 65 per microbial genome and USD 130-600 per multiplexed metagenomic clinical sample), but fully loaded costs are significantly higher (currently about USD 3,400 per clinical whole genome test, including instrument amortization, bioinformatics, storage and interpretation) (“Next-generation sequencing in infectious-disease diagnostics: Economic, regulatory, and clinical pathways to adoption,” 2026). The proportion of capital costs to per-sample cost is very small. For surveillance, the cost per sample for whole genome sequencing (WGS) ranged from USD 1.2 to 4.3 times that of traditional approaches, but break-even analyses indicated that it would cost the same or less per sample than traditional methods for preventing 0.2-1.1% of reported salmonellosis cases (Alleweldt et al., 2021), and a review of economic evaluations reported single-test costs of USD 72-470 and concluded that there was evidence of economic viability, particularly for foodborne illness and hospital infection control. Reagent and laboratory costs of metagenomic testing of postoperative CNS infection, however, are higher than that of culture, and the net cost-effectiveness has yet to be determined. There are few economic evaluations of laboratory tests, with many rapid and point-of-care tests being introduced without cost-effectiveness studies, and with no universally accepted methods for economic evaluation of diagnostics. There have been more sequences than they can analyze. Laboratories require lab space, digital space, computing and storage, expertise in data quality management and information technology systems, and pipelines and wet-lab methods and platforms. Limited skilled staff, limited infrastructure and limited bioinformatics capacity are major problems in low-resource environments and infrastructure, skilled staff and computing activation costs are not reflected in reagent cost.

The performance of reported mNGS is not guaranteed, either. The weighted F1 scores of 122 laboratories which were assessed with lower respiratory infection reference panels ranged from 0.20 to 0.97, with most false positives (68.56%) occurring in the wet laboratory and most false negatives (76.18%) due to loss of microbial sequence in the wet laboratory (“Assessing the quality of metagenomic next-generation sequencing for pathogen detection in lower respiratory infections,” 2023). In a like survey of the CSF testing in 127 laboratories, most false positives (83.43%) were attributed to experimental contamination (“A multicenter evaluation of metagenomic sequencing for pathogen detection in central nervous system infections,” 2026). The results of interlaboratory studies indicate that the choices of protocol add bias and variable robustness. Mitigation is based on validated extraction and library construction protocols, reference materials, proficiency testing, external quality assessment, controlled and documented bioinformatics, performance specifications (e.g., base-calling accuracy and reproducibility >99.9% for whole genome sequencing under CLIA) and standardized protocols in compliance with ISO 15189 and ISO 20397. The lack of transparent algorithms and defined interpretative thresholds in the dry laboratory also prevents independent verification and ESCMID is establishing consensus quality criteria for clinical metagenomics. Contamination is the most known cause of false positive results of the nucleic acid methods. Amplicons, other specimens, positive controls, reagents, water, disposables and carryover can all result in false results; particularly in the case of broad-range 16S PCR, false virus discoveries have been attributed to contamination of reagents and extraction controls with parvovirus-like sequences, which have been found in extraction controls. Prevention is based on multiple quality programmed: laboratory design and unidirectional flow, staff training, inactivation of amplicons, environmental monitoring, monitoring of the positivity rate, risk assessment and contamination-event plans. Stricter controls and clinical correlation are required with more sensitive technology. Diagnostic technologies are typically created and tested in well-equipped labs, however their conditions are different where they are needed. Tests intended for resource-limited settings must be usable in remote locations by people with little laboratory training, and molecular and cytology-based methods in high-resource settings typically depend on expensive equipment. This has driven interest in isothermal amplification, affordable instruments and point-of-care approaches supported by digital tools or AI. Laboratory validation of a low-cost system does not establish that it will work in a district hospital, where staffing, power, supplies and workflow differ, so suitability must be confirmed locally. Performance should therefore be stated together with the setting, operator training and equipment: a simpler, less sensitive test that is dependable and accessible may do better Integration of Molecular Diagnostics, Proteomics and AI

Conventional culture is now more often considered complementary to molecular diagnostics, as they address different questions: molecular diagnostics identifies genetic material and genotype, and conventional culture identifies if there is living organisms and allows phenotypic testing. In situ hybridization, microarrays and nucleic acid amplification accelerate identification, and for positive blood cultures, results are returned for panels including VERIGENE in less than 2 hours and for the FilmArray blood culture panel its ability to detect over 25 pathogens and four resistance genes means results are available within 1 hour of positivity (“Rapid identification of pathogens from positive blood cultures,” 2014). In routine clinical samples, 16S rRNA gene NGS, combined with culture, improved the diagnostic sensitivity in samples with confirmed culture infections,

in >60% of the cases (confirming the culture in approximately 21% and improving detection in approximately 40%) ("Performance of 16S rRNA gene next-generation sequencing and the...," 2024). A consistent practical model is beginning to emerge: rapid PCR for early rule-in diagnosis, and culture and susceptibility testing for confirmation, de-escalation and dosing. Molecular assays are useful as an adjunct to blood culture as they improve detection and time to actionable information, although not used as a substitute due to their limited sensitivity and limited validated targets. Two types of integration emerge. The first is MALDI-TOF protein-based identification and molecular confirmation: MALDI-TOF identifies faster than biochemical tests and 16S rRNA sequencing is used to resolve discordant or ambiguous identifications, and a paradigm of MALDI-TOF first, sequencing second was proposed for identification of dermatophytes; 16S rRNA sequencing was combined with MALDI-TOF for roughly 99% of dermatophyte identifications, and a paradigm of MALDI-TOF first, sequencing second was proposed. Combining PCR discrimination and resistance detection with low operating cost, amplified nucleic acids are detected in the second, multiplex PCR amplifies many targets and mass spectrometry discriminates the products. In 133 clinical samples (28 species identified versus 12 by real-time PCR), nucleic-acid MALDI-TOF had 89.29% sensitivity and 97.14% specificity for nontuberculous mycobacteria, while a 23-plex PCR coupled to MassARRAY identified 11 sexually transmitted pathogens at the same time. The practice of using complementary is better supported by evidence than full replacement.

The final identification and susceptibility have traditionally required approximately two days after a blood culture is positive. Rapid identification with stewardship reduced the time from culture positivity to final results from approximately 47 to approximately 24 hours, with costs to hospitals reduced. Assays that combine identification and susceptibility testing are available, such as PCR with high resolution melt, multiplex TaqMan qPCR and biosensor platforms reporting both identification in approximately 45 minutes and phenotypic susceptibility directly from the urine sample for UTI. Total laboratory automation and staged "funnel" designs are based on a broad automated phenotypic screening, followed by molecular assays for selected positive or unresolved cases; "FAST microbiology" approaches include adding MALDI-TOF or multiplex molecular assays to blood culture, as soon as it is positive, and implementing structured communication between the laboratory and the clinician. To overcome lack of standardization, standardized sample-to-answer metagenomic pipelines (extraction, host depletion, dual DNA/RNA library preparation and sequencing) with a roughly 24-hour turnaround are available. There is no such thing as concordance and it is best to approach discordance as a warning flag for structured interpretation. The principal issue in microbiology is the genotype-to-phenotype gap: a gene that confers resistance is reported by a rapid platform and it is not confirmed by phenotypic testing, or the other way around. It is recommended to classify discordance by cause (technical, threshold related, population related, database related, temporal or mechanistic), validate, adjudicate with smallest independent step required and interpret in clinical context. Illustrative examples from other areas demonstrate that the confirmatory test is not necessarily the proper test, and that discrepant-analysis designs are distorting tests of comparisons. In recent literature, molecular diagnostics, proteomics and AI are being considered as a single system, and single-omics analyses often have limitations in capturing the complexity of disease. Proteins and post-translational modifications are more relevant to signal execution than DNA or RNA, so proteomic biomarkers are still relevant. For high-dimensional, heterogeneous multi-omics data, a range of techniques requiring AI, such as feature selection, autoencoders, graph neural networks and federated learning (with privacy) must be employed. Molecular maps generated by Mass spectrometry imaging are correlated with histology and their molecular profiles are linked to tissue structures using Digital Pathology and AI. Combined use of NGS and multimodal evaluation for culture-negative infections: targeted NGS for etiological diagnosis in spinal infections, molecular tools as adjunctive tools to imaging in culture-negative endocarditis, and molecular diagnostics for culture-negative and polymicrobial prosthetic joint infections. Genomic, transcriptomic and proteomic data on platforms like PATRIC are mapped to phenotype metadata so that ML can make a connection between genomic features and resistance. The majority of these are reviews, proposals, or prototypes (some trialed with synthetic data) and therefore the promise of the field is more well documented than the field's demonstrated clinical performance. The core conclusion of the literature is accelerated diagnoses, if combined with stewardship, offer benefits that are mainly associated with the latter. With the use of rapid diagnostic tests and antibiotic stewardship, the time to effective therapy for antibiotic-resistant Gram-negative bacteremia was reduced, all-cause mortality was reduced at 30 days (21% to 8.9%), and hospital costs were lowered by approximately USD 26,000 for each surviving inpatient ("Rapid microbiological diagnostics for sepsis in critical care," 2026). A more recent review found that the odds ratio for death compared with conventional therapies was 0.72 and time to optimal therapy was reduced by almost 30 hours (Peri et al., 2024). The relevance of counterweights: increased timeto-targeted therapy and increased clinical confidence were seen with accelerated diagnostics in a real-world "FAST microbiology" evaluation, but this was not the sole driver of overall and 28-day mortality, which was largely due to severity of illness, age and comorbidity. Quick diagnosis can be a precursor to delayed clinical response. However, there are risks (algorithmic bias, calibration drift, software error, pre-analytical variability) and costs (initial costs, space, downtime, reliance on manufacturers) associated with automation.

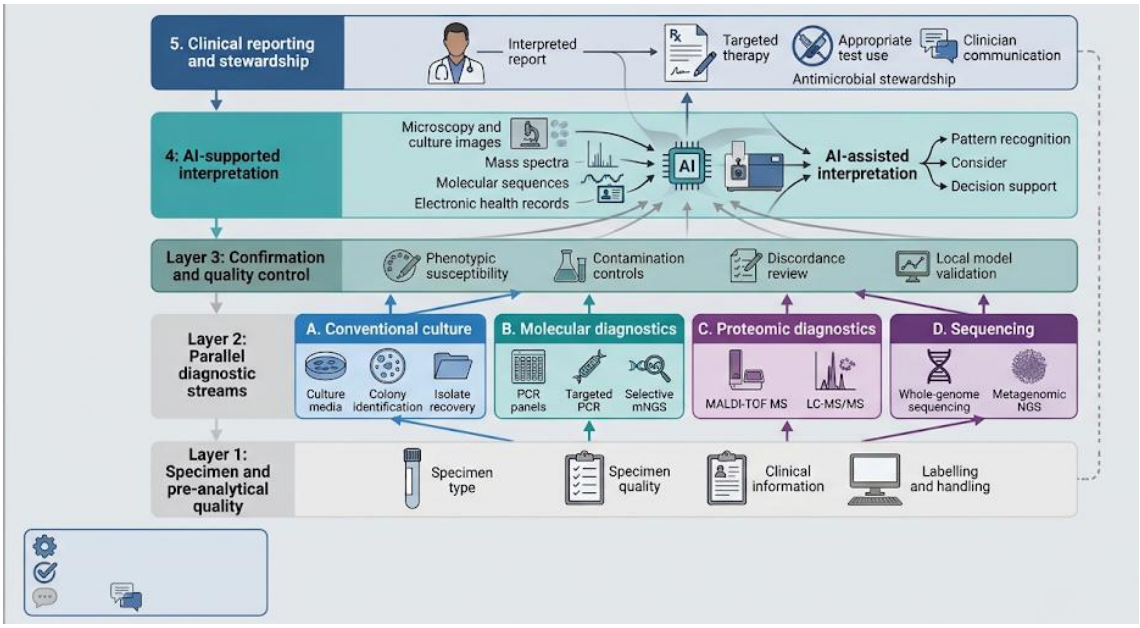


Figure 3. Proposed integrated diagnostic framework.

Layered schematic showing specimen information and pre-analytical quality at the base; parallel culture, molecular (PCR panel, targeted or metagenomic NGS) and proteomic (MALDI-TOF, LC-MS/MS) streams in the middle; an AI-supported interpretation layer drawing on images, spectra, sequences and electronic health record data; and clinician-facing reporting linked to antimicrobial and diagnostic stewardship at the top. Confirmation and quality-control checkpoints (phenotypic susceptibility, contamination controls, discordance review, local model validation) are placed between layers, with a feedback loop from outcomes to test selection. Original conceptual diagram to be prepared by the authors; it is illustrative and not a clinically validated protocol.

Clinical Applications

Blood stream infection and sepsis. No single rapid and accurate diagnostic test is available to support the recommendation to start empiric antibiotics within 1 hour of the recognition of sepsis and septic shock. Rapid molecular techniques (PCR, LAMP, microarrays, sequencing) allow for the identification of pathogens and resistance directly from blood, thereby decreasing the time for diagnosis and the need for using broad-spectrum antibiotics. Newer techniques are based on T2 magnetic resonance for detection of pathogens, morpho kinetic cellular analysis for susceptibility testing, or host biomarkers like C-reactive protein and procalcitonin. Biosensors and peptide-functionalized Nano systems are being developed using nanotechnology for capture and detection of low concentration pathogens, but many applications are at the review or developmental stage. For patients in the ICU, good sampling of specimens, prompt empiric therapy, therapeutic drug monitoring and source control are highlighted. Respiratory tract infection. Acute respiratory viruses are now detected by nucleic acid amplification tests, while syndromic panels detect the common causes of upper and lower respiratory infection quickly and at the same time, and have been shown to have an impact on antibiotic withholding. In primary care, point-of-care CRP testing is likely to decrease antibiotic use for ARI without impacting recovery and CRP and procalcitonin assist with antibiotic duration in pneumonia. In the more challenging lower respiratory specimens, lavage fluid is the best specimen for mNGS. Infection of the CNS. Meningitis, encephalitis and abscess are time sensitive emergencies, where the delay in antibiotic or antiviral treatment will lead to complications. There are multiple scenarios of using PCR on CSF over culture or antigen detection, including when it is more sensitive, more rapid and is suitable for multiplex meningoencephalitis panels, which reduce the TAT, but have suboptimal analytical performance that may require confirmation; finally, mNGS is proposed when conventional evidence is lacking. Use of host response biomarkers was shown to add value: When CSF leukocyte count was indeterminate, a model based on CSF CRP, IL-6 and IL-1β discriminated bacterial meningitis nearly perfectly (AUC 0.99) (Olie et al., 2024). These data were obtained from single cohorts and require confirmation. Urinary tract infection. High resistance to tetracycline (69.1%), sulphonamides (59.3%), quinolones (49.4%) and beta-lactams (36.9%) was found and UTIs account for approximately 25-40% of primary care antibiotic use, with uropathogenic E. coli being the predominant cause ("Virulence factors and antimicrobial resistance of uropathogenic Escherichia coli," 2021). Multiplex PCR, mass spectrometry and NGS also enable detection of low count or atypical pathogens and resistance determinants which is superior to culture, particularly in complicated and recurrent infection, but for now their clinical significance is uncertain until cure rates and symptom response is known. A nanopore metagenomic platform had a sensitivity of 89.9% and a specificity of 100% when tested against a composite standard in 213 urine samples

("Clinical evaluation of metagenomic next-generation sequencing in unbiased pathogen diagnosis of urinary tract infection," 2023).

HAP and Fungal infection. HAPI (HCAI) (urinary, bloodstream, lower respiratory, surgical site) are associated with increased mortality, increased cost, increased length of stay; antimicrobial resistance is greater in device associated infections. Hand hygiene, environmental cleaning, screening, surveillance and stewardship are the bases of prevention. Candida auris is an example of a multidrug-resistant organism, one that spreads from patient to environment and is rarely identified by standard, easily-accessed methods, and requires a reliable method of identification, such as MALDI-TOF or sequencing (D1-D2 28S, ITS); in outbreak settings, laboratories have used in-house real time PCR in combination with MALDI-TOF for screening.

Investigation and surveillance of outbreaks. Whole genome sequencing, coupled with patient location data, identified an outbreak of carbapenem resistant KP in a hospital cluster from a patient who was discharged weeks prior, demonstrating how silent colonization is able to allow strains to disseminate. mNGS has been applied to undiagnosed syndromes during outbreaks (including novel viruses), and using advanced molecular methods to detect virus presence in conventionally negative patients (coordinated sentinel surveillance) has been suggested. There is no routine surveillance for most invasive fungal diseases and weak laboratory capacity to respond, especially in Africa, where building surveillance capacity and establishing regional data hubs, and One Health approach are called for. Infection prevention and stewardship. Two sides to the same issue: IPC and antimicrobial stewardship – prevention is the first rule of antimicrobial stewardship, since they are often the medicines that lead to resistance that are needed to treat HAIs. The majority of hospital stewardship programmed have demonstrated favorable clinical and economic results and a 5-year before-and-after study demonstrated that the programmed were associated with substantial reductions in C. difficile infection, ventilator-associated pneumonia, and central line-associated bloodstream infection and with a decrease in the use of broad-spectrum antimicrobials. There is significant role for pharmacists and nurses and integrated multimodal programmed seem to be best, though many outcome data are based on before and after or single system designs.

Implementation Challenges and Research Gaps

Implementation, not effectiveness, is the main barrier. The largest challenge in tackling HAIs and resistance is putting evidence-based measures into practice. Priority-setting exercises highlight organizational, socio-economic and behavioral barriers and call for implementation research and collaboration with the social sciences. In low- and middle-income countries the barriers are structural, with a lack of trained staff, inadequate microbiology support, lack of governance and leadership, inadequate funding, restricted access to technology and expertise, and interrupted supply chains; merely transferring interventions from higher income countries to LICs is not effective. In settings of wealth and community, multidisciplinary collaboration, training opportunities and uptake are limited, and time-constrained primary-care physicians have low uptake. Machines, repair and personnel. It is not technology alone that is limiting the use of diagnostic equipment in rural and resource-constrained environments, but the inability to maintain and operate the equipment and cover costs for spares and manufacturer's contracts: unreliable electricity, lack of maintenance personnel and funds, expensive spares and manufacturer contracts dependent on foreign technicians. In one study of resource-poor hospitals, 72% of broken equipment was repaired using locally available materials, which would suggest a focus on biomedical technician assistants, and a computerized maintenance management system was sustainable with appropriate training in Burundi ("Evaluating a computerized maintenance management system in a low resource setting," 2021). Possible solutions are mobile laboratories and those using renewable energy, scholarships and telehealth training, e-learning and simulators and task shifting. While operating costs can be reduced through life cycle cost analysis, preventive maintenance, and condition-based maintenance, and through optimization, including equipment selection and replacement timing-reported savings (typically 10% to more than 30%) are mostly the result of model validations or specific projects. Design of study and quality of evidence. Diagnostic accuracy designs are heterogeneous and are inferior in their ability to improve the estimation of the accuracy. Validation should be intended (targeted) to the use to which it will be applied; should be in the cohort design with complete verification and adequate sample sizes; and be approached with caution when dealing with claims based on case-control or convenience sampling. Digital health technologies need a three-part framework of verification, analytical validation and clinical validation. Standardization and interoperability of data. Large-scale research and AI are hindered by heterogeneous data. The backbone of laboratory terminology, LOINC is used in national policies around the world and in US certified health IT along with SNOMED CT and UCUM, but standards are inconsistently applied, information is lost in translation (e.g., specimen source, units), and multilingual translation is not straightforward. Common data models (OMOP), HL7/FHIR messaging, IHE integration profiles and retrospective harmonization frameworks are being adopted and laboratory tests are being mapped to LOINC using LLM with 81-90% accuracy with experts in the role of verification ("Clinical laboratory terminology standardization for semantic interoperability using a large language model–based agent," 2026). Regulation, quality management and governance. The CLIA and ISO 15189 are established models that encompass technical, process and personnel needs, and the College of American Pathologists added 18 accreditation requirements for NGS (Jennings et al., 2014). For AI, proposals are CLIA-like accountability, calls for algorithm registration and assessment by

oversight boards (clinical value, fairness, usability, transparency and regulatory status), and continuous monitoring and repeated local validation instead of a single external validation. The overarching theme is Governance: AI should be incorporated into the laboratory quality management system, with clear accountability throughout the lifecycle. Many of the sources consist of proposals and commentaries, and focus on what governance ought to be rather than on better outcomes. Demonstrating value. Laboratories are still being judged almost exclusively on throughput and charged for the test performed, meaning that not much credit is given to patient outcomes. Value is outcome in relation to cost, with measures of diagnostic impact along the care pathway, health technology assessment for diagnostics (e.g. GRADE) and models like “Clinical Lab 2.0”. Evidence of effectiveness and cost-effectiveness of diagnostic services is limited, and the "third translational gap" remains when it comes to implementing evidence and demonstrating benefit in practice.

Factors that hinder the introduction of novel technologies. Limited uptake due to lack of: demonstrated clinical utility, regulatory affairs, reimbursement, workflow integration, interoperability, staff skills, ethical and liability concerns, and unrepresentative evidence. Stakeholder partnerships and designing innovations for dissemination through staged pathways to validation (analytical, clinical, clinical utility, regulatory) and designing for accelerated translation have been proposed but their impact on adoption has not been tested.

Future Perspectives

Molecular POCT is shifting from the promise to practice: Simplified NAA, Direct amplification, Visual or colorimetric readout, Non-instrumented heating and Smartphone platforms. Beyond nucleic acids, CRISPR-based diagnostics are broadening the scope of sensing to proteins and small molecules, while low-cost designs converge on the ASSURED criteria: electrochemical biosensors, under USD one paper-based devices, integrated microfluidic systems that can test SARS-CoV-2 in 28 minutes for USD 9.50 per test (“An integrated microfluidic platform for nucleic acid testing,” 2024), and power-free extraction with lyophilized LAMP chemistry using heaters for less than USD 100. Challenges include not just technical: sample processing needs for CRISPR; challenges in translating LAMP from lab to field; few commercial nucleic acid solutions for biosensors; regulatory approval; data privacy; and a lack of standardization of reporting of performance metrics. The first improvements that will be seen with AI will be those of image-based automation (plate reading, Gram stain interpretation, automatic release of negative cultures) within the context of a complete total lab automation platform like Kiestra or WASP Lab. Multimodal AI is expected to integrate genomic, transcriptomic, proteomic, imaging and electronic health records data in real-time, interpretable decision support, such as digital twins and data from wearable devices; most of these are prototypes or simulations for which clinical value has not been established. Algorithmic research is becoming increasingly focused on graph neural networks, transfer learning, federated learning and hybrid architectures, and on co-governance models such as regulators, developers, clinicians, patients, and ethicists. In the field of proteomics, harmonization and multicenter validation are the practical way to achieve reproducible results – eleven international sites generated reproducible results with a proteomics workflow (“Standardization and harmonization of distributed multi-center prototype analysis supporting precision medicine studies,” 2020); standardized pathology scoring; and in preclinical research, harmonized protocols had a significant impact in reducing between-laboratory variability. Six priority domains were identified by a global survey of unmet needs: AI integration, Genomics capacity, Global harmonization, Decentralized diagnostics, Biomarker validation and implementation science (“Bridging innovation and implementation in laboratory medicine,” 2026). Technology is evolving faster than evidence, regulation and infrastructure to put it into use, the sources agree, and near-term growth requires regulatory clarity, operational readiness, procurement, reimbursement and a proven clinical benefit—and several forecasts are expectations not performance.

Conclusion

Conventional culture is the bedrock of clinical microbiology as it is standardized, inexpensive, and is the only method to obtain viable isolates, phenotypic susceptibility, resistance surveillance and outbreak investigation. There is no doubt that its boundaries are not elastic: it is slow, is not sensitive to antibiotics after exposure, and cannot recover fastidious and slow growing or non-culturable organisms. These are inherent in the growth principle which is why culture independent and accelerated method has emerged.

Rapid molecular diagnostics (real time PCR, syndromic panels, isothermal assays, targeted and metagenomic sequencing) provide speed, sensitivity and breadth, quick access to resistance data, and the ability to identify organisms for which culture fails to detect, but they require a sophisticated interpretation, have limited targets, and are susceptible to contamination and poor laboratory performance. While the tandem mass spectrometry prototyping and mass-spectrometric resistance assays are still largely research tools, proteomic technologies, such as MALDI-TOF, have become the staple of identification tools for cultured isolates and positive blood cultures at very low cost per test. AI can improve image reading, interpretation of images, analysis of sequences and laboratory management, but requires training data; transfer poorly between instruments and sites; and needs to be explained, validated locally, monitored, and professionally accountable.

The technologies are complementary elements of a single pathway: culture and phenotypic susceptibility as the confirmatory backbone; mass spectrometry for a rapid confirmation; molecular technologies for rapid rule-in and broad detection; sequencing for unexplained or culture-negative disease; and AI as a tool for data-rich or subtle interpretations beyond the scope of human capacity. The main lesson that can be gleaned from the central practical lesson is that the faster you do something doesn't make things better-benefit is greatest when the fast results are communicated quickly, acted upon within the framework of diagnostic and antimicrobial stewardship, and some real-world studies have failed to demonstrate any mortality effect when severity and comorbidity dominate the picture.

Therefore, clinical validation and quality assurance is needed for integration. There is extensive variation between laboratories, imperfect reference standards hinder comparisons, genotype-phenotype discordance cannot be avoided, and AI models drift. Future research priorities include prospective, multicenter studies with patient-centered outcomes and cost-effectiveness endpoints; standardization of wet laboratory and bioinformatic workflows, with reference materials and external quality assessment; improving databases and standardizing reporting of sequencing and MALDI-TOF; locally monitored and explained AI tools, with local validation; improving interoperability through shared terminologies; stewardship behavior and implementation science, and affordable, robust platforms and staff training for low-resource settings.

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