Review

Novel Assays and Biomarkers in Infectious Disease Detection: From Diagnosis and Prognosis to Therapeutic Monitoring and Cure Assessment

Mohammad Mahmoudi , Marifah Albalawi , Christiana Stanley , Jody Berry , Hazim Aljewari *

Posted Date: 20 March 2026

doi: 10.20944/preprints202603.1630.v1

Keywords: infectious diseases; diagnostics; biomarkers; nucleic acid amplification tests; metagenomic sequencing; point-of-care testing; therapeutic monitoring

 doi:10.20944/preprints202603.1630.v1

Review

Novel Assays and Biomarkers in Infectious Disease Detection: From Diagnosis and Prognosis to Therapeutic Monitoring and Cure Assessment

Mohammad Mahmoudi [1,2], Marifah Albalawi [1,2], Christiana Stanley [1], Jody Berry [1] and Hazim Aljewari [1,*]

[1] Now Diagnostics Inc, Springdale, AR, USA

[2] Ralph E. Martin Department of Chemical Engineering, University of Arkansas, Fayetteville, AR, USA

* Correspondence: hazim.aljewari@nowdx.com

Abstract

Infectious disease diagnosis remains central to clinical care, but current methods still have important limits. Clinical symptoms are often nonspecific, culture-based methods can be slow, serology depends on timing, and molecular tests may detect microbial material without always proving active disease. This review examines novel assays and biomarkers in infectious disease detection from a clinical perspective. It summarizes major diagnostic platforms, including advanced nucleic acid tests, syndromic panels, metagenomic sequencing, serological and antigen assays, point-of-care platforms, biosensors, and multi-omics approaches, and reviews pathogen-derived, host-response, and combined biomarker classes. It discusses how these tools can support diagnosis, prognosis, disease staging, therapeutic monitoring, and cure assessment. A central message is that analytical novelty alone is not enough: new assays must be accurate, timely, interpretable, and able to change patient management in real practice. Clinical symptoms and the physicians awareness of these remain critical along with the correct biomarkers. Translation is often limited by imperfect reference standards, limited external validation, poor standardization, workflow barriers, cost, and unequal access. Future priorities include stronger validation, simpler and standardized workflows, wider access through home (OTC) use, and better biomarkers for treatment response, cure, and relapse prediction.

Keywords: infectious diseases; diagnostics; biomarkers; nucleic acid amplification tests; metagenomic sequencing; point-of-care testing; therapeutic monitoring

1. Introduction

Infectious disease diagnosis remains one of the most important parts of clinical care [24,294] because treatment decisions, infection control [24], and public health [119] action all depend on timely and reliable test results [24,294]. In practice, however, diagnosis is rarely simple [1,2]. Clinical symptoms are often nonspecific [2], culture-based methods can be slow [24], serology depends heavily on the timing of the immune response [4], and molecular tests may detect microbial material without always proving active disease [7,213]. These limits show why infectious disease diagnostics still need major improvement [119,294].

The role of diagnostics has also become broader. Today, clinicians need more than a simple answer to the question of whether a pathogen is present. They also need tests that help identify the likely cause of illness [24], distinguish infectious from noninfectious inflammation [19], estimate severity and stage, follow treatment response, and assess cure or relapse risk [11,24]. This wider clinical role has increased interest in biomarkers from three main groups: pathogen-derived markers [12], host-response markers [15,17], and combined multi-marker signatures [11,20]. These approaches are especially valuable when direct pathogen detection is negative, delayed, or difficult to interpret [15,19,24].

 doi:10.20944/preprints202603.1630.v1

At the same time, the field is changing quickly [79,88,165]. Real-time and multiplex PCR [41,51], digital PCR [56], isothermal amplification [59], CRISPR-based assays [73], syndromic panels [79], metagenomic sequencing[88], improved antigen and antibody tests[108,109], point-of-care platforms [119,125], biosensors [136], and multi-omics approaches [165,166] are expanding what can be detected [79,88,166] and how fast results can be delivered [41,51,119]. Many of these tools offer clear advantages [79,88], but they also create new interpretation challenges [79,88]. A test that is analytically impressive is not automatically clinically useful [79,88], especially when colonization [79], contamination [79,88], residual nucleic acid [88], or host heterogeneity [17] can affect the result. Common microorganisms that are frequently carried by healthy individuals and may therefore be detected by molecular assays despite the absence of active infection are summarized in Table 1.

Table 1. Opportunistic pathogens commonly carried by healthy individuals that may confound molecular diagnostic tests.

Body Site	Potential Pathogen	Associated Infections	Healthy Population Carriage	Reference
Skin	Staphylococcus aureus	Minor skin infections to severe disease including sepsis	20–60% carriers in healthy individuals	[319]
Skin	Candida spp.	Cutaneous infections, vulvovaginal candidiasis, bloodstream infections	30–40% oral carriage in healthy adults	[320]
Skin	Streptococcus pyogenes	Pharyngitis; rheumatic fever	15–20% asymptomatic oral carriage	[321]
Oral cavity	Streptococcus mutans	Dental caries	40–80% carriage in healthy adults	[322]
Oral cavity	Actinomyces israelii	Abscesses and actinomycosis	~90% carriage in healthy children	[323]
Oral cavity	Fusobacterium spp.	Periodontitis and other systemic infections	37–86% carriage in healthy individuals	[324]
Upper respiratory	Haemophilus influenzae	Meningitis, pneumonia	4–35% carriage in healthy adults	[325]
Upper respiratory	Neisseria meningitidis	Meningitis	5–25% asymptomatic carriage	[326]
Upper respiratory	Streptococcus pneumoniae	Pneumonia and invasive pneumococcal disease	5–90% carriage depending on age	[327]
Upper respiratory	Legionella pneumophila	Legionnaires' disease (pneumonia)	Low carriage; 10–40% seropositivity	[328]
Gastrointestinal	Klebsiella pneumoniae	Pneumonia and opportunistic infections	5–38% stool carriage in healthy individuals	[329]
Gastrointestinal	Clostridioides difficile	Diarrhea and colitis	7–15% asymptomatic carriage	[330]
Gastrointestinal	Rabies virus	Fatal CNS infection	No healthy carriage	[331]

 doi:10.20944/preprints202603.1630.v1

Urogenital	Streptococcus agalactiae (Group B Streptococcus)	Neonatal invasive infections	15–30% carriage in adults	[332]
Urogenital	Gardnerella vaginalis	Bacterial vaginosis	26–50% carriage in healthy women	[333]
Urogenital	Chlamydia trachomatis	Urogenital infections; pelvic inflammatory disease	Often asymptomatic (≈70–80% women; ≈50% men)	[334]

For this reason, the central issue is not only innovation, but clinical usefulness [24,295]. New assays and biomarkers must be shown to work in the right patients, with the right specimens, and for the right clinical question [295]. Their value can be weakened by imperfect reference standards [285,286], limited external validation [292], poor standardization [292,295], workflow barriers [24,293], cost, infrastructure needs [292–294], and unequal access across settings [294,297]. In infectious diseases, the best diagnostic advances are therefore those that provide results that are accurate, timely, interpretable, and able to change patient management in real practice [24,119]. This review examines novel assays and biomarkers in infectious disease detection from that clinical perspective. It summarizes major diagnostic platforms, reviews key biomarker classes, and discusses how these tools can support diagnosis, prognosis, disease staging, therapeutic monitoring, and cure assessment. It also highlights the analytical [295], translational [24,292], and implementation [24,293,294] challenges that will shape the next phase of progress in the field [24,292–294].

2. Conceptual Framework for Infectious Disease Detection

2.1. Direct Versus Indirect Diagnostics

Infectious diseases can be diagnosed in two main ways: by finding the pathogen (or parts of it) in patient samples, or by finding the host response to that pathogen [1]. In real practice, diagnosis usually uses a mix of clinical findings plus lab tests, because symptoms alone are often not specific [2].

2.1.1. Direct Detection (Pathogen Nucleic Acids, Antigens, Toxins, Metabolites)

Direct detection means the test looks for the pathogen itself or something the pathogen makes [1]. This includes pathogen nucleic acids (DNA/RNA), antigens (pathogen proteins or polysaccharides) [1,2], toxins, and metabolites. Nucleic acid tests (NAATs) detect pathogen genetic material, most often using PCR-based methods. A key advantage is that NAATs can be fast and sensitive and do not require the organism to be alive (unlike culture). A key limitation is that NAAT results can be affected by contamination and by how well the assay is designed and controlled, so quality control is important [3]. Antigen tests detect pathogen structural components [4], and they are often built as rapid immunoassays [4,8]. A common advantage is speed and simplicity, especially for near-patient testing [8]. A strong example is cryptococcal antigen lateral flow testing, which has been validated across sites for diagnosing cryptococcal meningitis [10]. Toxin tests look for disease-causing toxins, which can be important because the presence of a toxigenic organism (or its DNA) is not always the same thing as active toxin-mediated disease. For Clostridioides difficile, guidelines describe diagnostic approaches that consider symptoms together with tests that detect toxin or toxigenic organisms, because interpretation matters [7]. Metabolite-based detection uses small molecules or volatile organic compounds (VOCs) that may come from the pathogen, the host response, or both. VOC/metabolite testing is promising as a noninvasive approach in some settings, but it is still less established than NAAT or antigen testing for most routine infectious disease diagnosis [9].

 doi:10.20944/preprints202603.1630.v1

2.1.2. Indirect Detection (Antibodies, Immune Signatures, Host Inflammatory Responses)

Indirect detection means the test measures the host response to infection rather than the pathogen itself [1]. This includes antibodies (serology), broader immune signatures (for example, gene-expression patterns), and more general inflammatory responses [4,6]. Serology is the classic indirect approach and is often based on detecting pathogen-specific IgM or showing a clear rise in pathogen-specific IgG between paired samples [4]. A key limitation is timing, because antibodies appear after a "window period," so very early infection can be missed by antibody tests. Serology can be very useful when the pathogen is hard to detect directly or when the direct-detection window has passed [4]. Host-response (immune signature) diagnostics aim to classify infection by measuring patterns in the host immune response, rather than identifying a specific organism [5,6]. These tests are often discussed as an adjunct to pathogen-based testing, especially when pathogen detection is slow, negative, or hard to interpret (for example, colonization vs infection) [5,6].

In summary, direct tests answer "is the pathogen (or a pathogen product) present [1]," while indirect tests answer "is the host responding in a way that fits infection[4,6]," and the best choice often depends on sample type and the timing of disease.

2.2. Biomarker Classes

A biomarker is a measurable sign that can show a normal process, a disease process, or a response to treatment [11]. In infectious diseases, biomarkers can come mainly from the pathogen, from the host response, or from both together in one combined score [15,16]

2.2.1. Pathogen-Derived Biomarkers

Pathogen-derived biomarkers are molecules that come from the microbe itself or are made directly because the microbe is present [12]. Common pathogen-derived biomarker types include pathogen DNA or RNA, pathogen proteins (antigens), toxins, and small molecules linked to microbial activity [12–14]. A key idea is that these biomarkers aim to give direct evidence that a pathogen is present in the sample being tested. In tuberculosis, many candidate biomarkers come from Mycobacterium tuberculosis molecules that can be found in patient samples, and these targets are being studied for better tests [12]. In fungal infections, antigen biomarkers such as galactomannan and beta-D-glucan are widely used, but their accuracy can change with the patient group, sample type, and test rules [13]. Small-molecule and metabolic signals can also act as pathogen-related biomarkers, because infection changes biochemical activity and can create measurable metabolite patterns [14]. Volatile organic compounds (VOCs) are another example of pathogen-linked biomarker profiles that may be measured from clinical samples, although routine clinical use is still limited in many settings [23]

2.2.2. Host-Response Biomarkers

Host-response biomarkers are measurements from the patient that reflect how the immune system and the body react to infection [15,16]. These biomarkers can include antibodies, inflammatory proteins, immune mediators, and host gene-expression patterns in blood or other samples [15–17].

Host-response markers can be helpful when pathogen tests are negative, slow, or hard to interpret, such as when colonization is possible [15,16]. Host gene-expression signatures are one important host-response biomarker type that can help separate bacterial from viral infection patterns in some cases [15,17]. A large comparison study showed that many different published gene-expression signatures exist, and their performance can vary across datasets and clinical groups [17].

 doi:10.20944/preprints202603.1630.v1

2.2.3. Composite and Multi-Marker Signatures

A composite or multi-marker signature combines more than one biomarker into a single score or set of outputs [11,15]. This approach is used because one biomarker alone is often not accurate enough for complex infection questions [15,19]. Some composite signatures use host mRNA panels, like the 29-mRNA InSep host response test for bacterial and viral infection prediction [18]. Some composite signatures use smaller gene sets, like the four-gene SeptiCyte Lab classifier designed to help separate sepsis from noninfectious systemic inflammation in critically ill patients [19]. Some composite signatures use multiple host proteins, such as a test using TRAIL, IP-10, and CRP to help distinguish bacterial from viral respiratory infections in adults [20]. A systematic review and meta-analysis also evaluated the combined TRAIL, IP-10, and CRP approach (MeMed BV) across multiple studies in febrile emergency department patients [21]. Composite signatures can also be based on cellular features, such as the IntelliSep cellular host-response test used for rapid sepsis risk stratification in the emergency department [22]. Even when composite tests look promising, they still need strong validation in the right patient populations and real clinical workflows [15,17]

2.3. Clinical Questions Addressed by Diagnostics

Diagnostic tests are used to answer specific clinical questions that guide patient care and public health actions [24]. Diagnostic stewardship helps make sure the right test is used for the right patient at the right time, so the test result can truly support a decision [25]. The first question is whether the patient's symptoms and test results support that an infection is present. This often needs a combination of clinical findings and laboratory evidence, because no single test is perfect in every setting [24]. Good diagnostic studies report sensitivity, specificity, and the reference standard clearly, so we can trust how well a test answers "infection present" [26]. Diagnostic stewardship also reduces testing in patients with very low pretest probability, which can lower false positive results and confusion [25]. The next question is which pathogen is causing the illness, because that can change treatment and infection control [24]. Syndromic molecular panels can rapidly detect many pathogens at once and can support faster decisions when used correctly [27]. Expert guidance stresses that rapid multiplex panels can help in critically ill patients but results still need careful clinical interpretation to avoid overcalling colonizers or contaminants [33]. Metagenomic next-generation sequencing can detect a broad range of organisms without a prior target list, which can help when routine tests are negative or incomplete [34]. A randomized trial in community-acquired pneumonia showed that syndromic molecular testing increased pathogen-directed treatment and shortened the time to targeted therapy compared with standard care [32]. Diagnostics are also used to estimate how severe the infection is and whether organ damage is happening [24]. In sepsis, severity is linked to organ dysfunction, and Sepsis-3 provides a widely used framework using SOFA-based organ dysfunction to define sepsis and identify higher-risk patients [28]. For chronic viral infections, laboratory markers can reflect disease activity and stage, not only the presence of infection [24]. In HIV care, plasma HIV-1 RNA (viral load) and CD4 counts are key measurements used to assess disease status and guide management over time [29]. Another key question is whether treatment is working, because this supports continuation, change, or stopping of therapy [24]. In HIV, viral load is the most important indicator of response to antiretroviral therapy, and guidelines give specific timing for monitoring [29]. In tuberculosis, repeated microbiology testing during treatment is used to track response, and WHO guidance describes how monitoring can differ between drug-susceptible and drug-resistant disease [36]. Time to sputum culture conversion has been studied as an early marker linked to treatment outcomes in multidrug-resistant tuberculosis cohorts [38]. Biomarkers for treatment monitoring are an active research area because faster and more reliable response markers could improve care and trials [37]. Diagnostic stewardship studies in pneumonia also show how faster pathogen results can support earlier, more targeted therapy decisions in real workflows [32]. Finally, clinicians often need to know if cure has been achieved, which usually requires a clear outcome definition [31]. For hepatitis C, guidance states that undetectable or unquantifiable HCV RNA 12 weeks or longer after treatment is an SVR, which is consistent with cure [30]. CDC clinical guidance

 doi:10.20944/preprints202603.1630.v1

also notes that hepatitis C can be cured in most cases with direct-acting antivirals, reinforcing cure as a realistic endpoint [40]. For tuberculosis, WHO definitions provide standardized outcome categories, including "cured" and "treatment completed," based on bacteriological evidence and program reporting rules [31]. The WHO TB Knowledge Sharing Platform summarizes newer outcome definitions that allow assigning cure or treatment success at treatment completion while noting differences in monitoring methods for DS-TB and DR-TB [35]. For some sexually transmitted infections, guidelines include "test-of-cure" in selected situations, showing that cure assessment can be condition-specific [39].

3. Novel Assay Platforms for Infectious Disease Detection

3.1. Nucleic Acid-Based Assays

3.1.1. qPCR and Multiplex PCR

Quantitative real-time PCR (qPCR) detects and amplifies pathogen DNA or RNA and measures the signal during the reaction. qPCR uses fluorescent chemistry so the instrument can read the amplification in real time [41,42]. The cycle threshold (Ct) value is the cycle number where the signal rises above background, and it is linked to how much target is in the sample.

qPCR can be used for qualitative results (detected or not detected) and also for quantification when standards and controls are used [41,43]. qPCR is widely used because it is fast, sensitive, and specific when the assay is well designed [41,42]. qPCR results can be affected by poor sample quality, inhibitors in the specimen, and contamination, so controls are essential. Good practice includes negative controls, positive controls, and internal controls to check extraction and inhibition [41,43]. The MIQE guidelines describe what information should be reported so qPCR results are transparent and reproducible [44,45]. Clinical laboratories also follow method validation, quality assurance, and result interpretation guidance for infectious disease NAATs [46].

Multiplex PCR tests more than one target in the same reaction, so it can detect several pathogens or genes at once. Multiplex PCR can save time and sample volume compared with running many single-target tests [47]. A common use is respiratory virus testing, where many viruses can cause similar symptoms and quick differentiation is helpful [49,50]. Multiplex design is harder than singleplex because primers and probes can interact and compete, which can reduce sensitivity for some targets. Multiplex assays also need careful optimization to avoid cross-reactivity and to keep each target working well [47]. Because multiplex tests are complex, strong analytical validation and ongoing quality control are important [48]. Evidence summaries show that commercial multiplex PCR systems for respiratory viruses are generally accurate for viral detection [49].

Clinical evaluation studies also show that multiplex real-time PCR can detect more respiratory viruses than some older methods in real practice [50]. A systematic review and meta-analysis found that rapid sample-to-answer multiplex PCR can reduce time to results and may improve clinical process outcomes in hospitalized patients [51].

3.1.2. Digital PCR (dPCR)

Digital PCR (dPCR) is a PCR method where the sample is split into many small reactions before amplification [52,55]. After PCR, each small reaction is read as positive or negative, and the total pattern is used to estimate how many target copies were in the original sample [52,55]. This makes dPCR a "counting" method that can give absolute numbers without needing a standard curve [52,55,56]. dPCR can be useful when the target level is very low, or when small differences in copy number matter. dPCR can also be useful when inhibitors or imperfect PCR efficiency make quantification by qPCR harder [55,56]. In infectious diseases, dPCR has been reviewed as a tool for viral, bacterial, and parasitic diagnosis, including situations where high sensitivity and accurate quantification are needed [56]. dPCR needs careful design and reporting, and the digital MIQE guidelines describe what should be included so results can be checked and repeated [53,54]. The term

 doi:10.20944/preprints202603.1630.v1

"digital PCR" became widely known after the 1999 paper by Vogelstein and Kinzler, and later reviews describe how the method developed over time [52,57]. Even with its advantages, dPCR can be limited by cost, platform availability, and workflow demands in routine laboratories [55,56].

3.1.3. Isothermal Amplification Methods such as LAMP and RPA

Isothermal amplification methods amplify nucleic acids at one constant temperature, so they do not need a thermal cycler [59,64,65]. This can make testing simpler and faster in settings with limited equipment [59,64,65]. Loop-mediated isothermal amplification (LAMP) was introduced as a method that amplifies DNA under isothermal conditions using specially designed primers and a strand-displacing polymerase [58,59]. LAMP can produce results quickly and can be read with simple detection formats, which supports near-patient or field use in some settings [59,60]. WHO has issued policy guidance for TB-LAMP and supports its use in defined ways for pulmonary tuberculosis diagnosis [60]. A systematic review and meta-analysis evaluated TB-LAMP accuracy and found it can perform better than smear microscopy and can be considered as an alternative in some settings, while noting study limitations and heterogeneity [61]. RT-LAMP has also been evaluated for SARS-CoV-2, including multisite clinical work on extraction-free workflows that aim to simplify sample preparation [62]. Like other amplification tests, LAMP can be affected by assay design and contamination risk, so controls and good workflow practices are important [59,61].

Recombinase polymerase amplification (RPA) is an isothermal method that uses recombination proteins and polymerase activity to amplify targets at low and constant temperature [63,64].

RPA can be fast and can work with minimal equipment, which supports point-of-care style testing concepts [63–65]. Reviews describe RPA as operating around body-like temperatures and producing amplification in short time frames for many targets [64,65]. A dedicated review summarizes RPA principles, detection formats, and infectious disease applications for pathogen detection [66]. Another review discusses RPA for point-of-care diagnosis of neglected tropical diseases and highlights use cases in low-resource settings [67]. RPA still faces practical limits such as primer and probe design demands and the need for strong validation to avoid nonspecific amplification [64,66,67].

3.1.4. CRISPR-Based Diagnostics

CRISPR-based diagnostics use a guide RNA and a CRISPR enzyme to recognize a matching DNA or RNA sequence in a sample [73,78]. After the target is recognized, the enzyme can cut reporter molecules and create a measurable signal. Many CRISPR diagnostic tests use this "collateral cleavage" effect, where the enzyme cuts many reporters after it binds the correct target [68,69,73].

Cas13 systems mainly target RNA, and target binding can activate non-specific cutting of nearby RNA reporters [68,73]. Cas12 systems mainly target DNA, and target binding can activate non-specific cutting of nearby single-stranded DNA reporters [69,73]. These collateral reactions allow signal amplification at the detection step, which can increase sensitivity [68,69,73].

Most CRISPR diagnostic workflows still use a nucleic-acid amplification step before CRISPR detection, especially for low viral or bacterial loads.[72–74]. The platform called SHERLOCK showed sensitive nucleic-acid detection using Cas13 and isothermal pre-amplification [68,72].

Later work added multiplexing and portable readouts by combining Cas enzymes and additional signal amplification strategies [70,73]. Field-deployable CRISPR-Cas13 testing was also demonstrated for viruses such as Zika and dengue in patient samples [71].

CRISPR diagnostics can be read by fluorescence, lateral flow strips, or other simple signal formats [72–74]. A detailed step-by-step protocol paper explains how to design guides, set up pre-amplification, and run Cas12 or Cas13 detection with common readouts [72].

During the COVID-19 pandemic, Cas12 and Cas13 assays were clinically validated for SARS-CoV-2 detection [75,76]. A Cas12-based assay showed rapid detection of SARS-CoV-2 with a lateral flow style readout [75]. A Cas13-based SHERLOCK assay was also clinically validated using

 doi:10.20944/preprints202603.1630.v1

respiratory swab samples [76]. A one-pot format called STOP simplified the workflow by combining steps into a more streamlined assay design [77].

Major challenges for CRISPR diagnostics include simple sample preparation, avoiding contamination, keeping sensitivity high without complex equipment, and making results easy to interpret [73,74,78]. Another challenge is standardization, because performance can change with specimen type, extraction method, guide design, and amplification conditions [73,74].

Recent reviews emphasize that strong multi-center clinical validation and clear regulatory pathways are needed for broad routine use [73,78].

3.1.5. Syndromic Molecular Panels

Syndromic molecular panels are multiplex nucleic-acid tests that look for many pathogens at the same time from one sample. They are designed around a clinical syndrome, such as respiratory illness, diarrhea, sepsis, or meningitis [79,80]. These panels can shorten the time to pathogen detection compared with step-by-step testing [79,83]. They can also increase pathogen detection because they include targets that are not always ordered in routine testing [79,80]. Syndromic panels can support earlier clinical decisions when the result changes treatment, isolation, or antibiotic use [79,81,82]. The clinical value is highest when the test is ordered for the right patient group and the result is acted on quickly [81,82,84]. Diagnostic stewardship is important because broad testing can also create confusing results when pretest probability is low [81,82]. A common challenge is that panels may detect organisms that are colonizers, remnants of past infection, or contaminants rather than the true cause of disease [79,81]. Panels can also detect multiple organisms in the same sample, and the clinician must decide which one is clinically important [79,80]. False positive and false negative results can still occur, so results should be interpreted with the clinical picture and other lab data [79,86]. Some targets have lower sensitivity than others because multiplexing can reduce performance for specific organisms. Respiratory syndromic PCR panels are widely used for viruses and sometimes bacteria [79,80]. A systematic review and meta-analysis found that rapid multiplex respiratory testing reduces time to results and can improve some clinical process measures [83]. Bloodstream infection testing often uses rapid molecular methods on positive blood culture material or as panel approaches paired with stewardship support [79,84]. A meta-analysis found that rapid molecular diagnostics for bloodstream infections can improve outcomes, especially when combined with antimicrobial stewardship interventions [84]. Gastrointestinal panels can rapidly detect many enteric pathogens from stool samples [79,85].

Studies show that GI panel implementation can change healthcare use and prescribing patterns, but careful ordering is needed to avoid unnecessary testing [81,85]. Decision support tools can reduce inappropriate GI panel ordering and improve stewardship outcomes [82,87]. Central nervous system panels, such as meningitis/encephalitis panels, can provide rapid etiologic results from cerebrospinal fluid [79,86]. A diagnostic accuracy meta-analysis found good overall performance for the ME panel, but it also highlighted risks of false positives and false negatives that can change management [86]. In summary, syndromic panels can be very useful when they are targeted to the right patients and paired with stewardship and clear interpretation rules [79,81,82].

They should be used as part of a diagnostic strategy, not as a stand-alone answer to every question [81,82].

3.1.6. Next-Generation Sequencing and Metagenomic Diagnostics

Next-generation sequencing (NGS) reads many DNA or RNA fragments in parallel and produces large amounts of sequence data from a sample [88,89]. In infectious disease testing, NGS can be used as targeted sequencing, whole-genome sequencing of an isolate, or metagenomic sequencing directly from a clinical specimen [99]. Metagenomic next-generation sequencing (mNGS) is an “agnostic” approach because it does not start with one assumed pathogen target [88,89].

mNGS can detect bacteria, viruses, fungi, and parasites in one test if their nucleic acids are present in the sample. This is useful when routine tests are negative or when the infection may be

 doi:10.20944/preprints202603.1630.v1

unusual, mixed, or unexpected [88,89]. A major clinical strength of mNGS is that it can provide actionable diagnoses in difficult cases [90,91]. A well-known example is the use of unbiased sequencing to identify a treatable cause of meningoencephalitis when standard testing did not find the pathogen [91]. A larger clinical study also showed how metagenomic sequencing can help diagnose meningitis and encephalitis across many possible causes [90]. Clinical mNGS requires careful laboratory validation because it is a complex "wet lab + bioinformatics" test [92,93].

Guidance papers describe key steps for validating universal pathogen detection workflows and interpreting results [92]. Separate standards also describe how to validate and monitor the bioinformatics pipeline, because software choices can change results [93]. Recent work has also proposed using "operational value" measures to compare different clinical metagenomics approaches in a more standardized way [94]. Sample type and sample preparation strongly affect mNGS performance [88,89,98]. Many clinical specimens contain much more human DNA than microbial DNA, which can reduce sensitivity for pathogen detection. Studies have shown that methods to deplete human DNA can improve the fraction of microbial reads and increase detection sensitivity in some settings [98]. Contamination is another major issue, because low-level microbial DNA can come from reagents, the environment, or handling steps [97]. Because of these issues, negative controls and careful interpretation are essential, especially for low-biomass specimens [92,97]. Newer studies show that clinical mNGS can be validated for specific syndromes, such as respiratory infection testing. A Nature Communications study reported laboratory and clinical validation of an mNGS assay for respiratory virus detection and discovery with a turnaround goal of less than 24 hours [95]. A Lancet Microbe study evaluated a rapid respiratory metagenomics service in the ICU and assessed feasibility, performance, and potential clinical impact [96].

NGS is also used outside direct specimen metagenomics [99,100]. Whole-genome sequencing of isolates supports outbreak investigation, infection prevention work, and detailed pathogen characterization [99]. Genomics is also becoming important for antimicrobial resistance surveillance, although routine adoption can be harder in low-resource settings [100]. Targeted NGS approaches can be useful when full shotgun metagenomics is not needed. For example, deep sequencing of bacterial 16S rRNA amplicons can help characterize polymicrobial infections when standard methods give limited information [101]. Clinical studies also reported that NGS can identify more organisms in polymicrobial samples than standard culture in selected settings [102].

Overall, metagenomic and other NGS approaches can expand the range of pathogens detected and can support faster or more precise decisions in selected use cases [88,89,94]. At the same time, these approaches need strong validation, careful contamination control, and clinically grounded interpretation to avoid misleading results [92,93,97].

3.2. Serological and Immunological Assays

Serological assays detect antibodies in patient samples to show current or past exposure to a pathogen [103–105]. Immunological assays measure parts of the immune response beyond antibodies, such as antigen-specific T-cell activity [116,117]. These tests are often used when direct pathogen detection is difficult, when the pathogen is no longer present in the sampled site, or when the goal is to measure immune status [103–105]. A key limitation is timing, because antibodies and cellular responses can take days to weeks to appear after infection [104,105].

3.2.1. Antibody-Based Serology

Serology commonly measures IgM and IgG antibodies, and the interpretation depends on the disease and the clinical setting. IgM can suggest recent infection in some diseases, but it can also be non-specific and may persist longer than expected. IgG can indicate past exposure, and paired samples can show a rising IgG level that supports recent infection when a clear increase is observed [104,105]. Cross-reactivity can occur when antibodies bind related pathogens, which can reduce specificity in some settings [103,105].

 doi:10.20944/preprints202603.1630.v1

3.2.2. ELISA and Chemiluminescent Immunoassays

ELISA is a common format for measuring antibodies or antigens using enzyme-linked signal generation. ELISA can be used for screening, confirmation, and research measurements when protocols and controls are well designed [106,107]. Chemiluminescent immunoassays are often used in clinical labs because they can be automated and can provide high-throughput testing. Chemiluminescent platforms are widely used for infectious disease serology, including SARS-CoV-2 antibody testing during the pandemic as one example [103,111].

3.2.3. Lateral Flow Assays and Rapid Tests

Lateral flow immunoassays are rapid tests that can be used near the patient and often give results in minutes. They can detect antibodies or antigens depending on the test design [108,109].

Their main advantage is speed and simple workflow, but performance can vary by product, specimen type, and viral load or antigen level [108,110]. Cochrane review summarized evidence for rapid antigen tests for SARS-CoV-2 and showed that accuracy depends strongly on timing and clinical context [110].

3.2.4. Antigen Detection Assays

Antigen tests detect pathogen components, such as viral proteins, and they can support early diagnosis when antigens are present [108–110]. Antigen tests are often faster and easier than nucleic acid tests, but they are commonly less sensitive, especially when pathogen burden is low [108,110]. Because of this, antigen test results often need careful clinical interpretation and sometimes confirmatory testing depending on the scenario [108,110].

3.2.5. Neutralization and Functional Antibody Assays

Neutralization assays measure whether antibodies can block infection or block a viral entry process in a test system. These tests are useful when the question is not only “antibody present” but “antibody can neutralize.” Standardization is important for comparing neutralization results across labs, and international standards can support better comparison [112].

3.2.6. Multiplex Immunoassays and Serosurveillance

Multiplex assays measure antibodies to multiple pathogens or multiple antigens in one run, which can support broader serosurveillance [113–115]. Multiplex bead assays can be used with dried blood spots, which can make large population studies easier in some settings [113,115]. Systematic reviews show that multiplex bead assays can support integrated serosurveillance, but assay harmonization and interpretation rules are still important [113]. Recent field studies have used multiplex serology to measure exposure patterns to multiple pathogens within the same communities [114].

3.2.7. Immunological Assays Beyond Antibodies

Some immunological assays measure T-cell responses to infection rather than antibodies. In tuberculosis, interferon-gamma release assays (IGRAs) are widely used to detect immune sensitization to Mycobacterium tuberculosis. Guidelines describe where IGRAs fit in TB diagnosis and how results should be interpreted with clinical risk and other tests [116,117]. Other assays for example, for joint replacement infections, measure host defensin levels such as the test from CD diagnostics who was acquired by Zimmer Biomet [118].

 doi:10.20944/preprints202603.1630.v1

3.3. Point-of-Care and Decentralized Testing Platforms

Point-of-care and decentralized testing means testing that can be done near the patient or outside a central laboratory [119,120]. These platforms aim to give fast results and support faster decisions [119,120]. Modern POCT also aims for good usability, low cost, and good data connectivity for reporting and follow-up [119,121].

3.3.1. Over the Counter Self Tests

Another class of testing is the self test. Made common by SARS-CoV2 pandemic, consumers want and need to be able to test themselves for major infectious diseases. Notably the Sfirst Syphilis self test (SST) was granted FDA clearance in 2024 [122]. For an authoritative review see [123].

3.3.2. Lateral Flow Assays

Lateral flow assays (LFAs) are simple strip-based tests that can detect antigens or antibodies in minutes [108,109]. LFAs are popular for decentralized testing because they are easy to run and do not need complex instruments [108,120]. LFAs can be used as screening tests, but accuracy can vary by product, specimen type, and timing of infection [108,110]. A large evidence summary for rapid antigen tests showed that sensitivity depends strongly on viral load and when the sample is collected [110]. Because LFA results can be hard to read by eye, digital reading tools can improve consistency, remove subjectivity and reduce user error [121,134]. Connectivity is also important so results can be stored, shared, and used for public health actions [119,121].

3.3.3. Microfluidic and Paper-Based Platforms

Microfluidic platforms move and process small fluid volumes in channels or droplets to automate steps like mixing, washing, and detection. These systems can combine sample preparation and detection in one small device to support fast testing outside the lab [125,126]. Microfluidics can also support multiplex testing by running many reactions in parallel on the same chip [124,125]. Paper-based microfluidics uses patterned paper to move fluids by capillary flow without pumps. Paper platforms are attractive for decentralized testing because they can be low-cost, light, environmentally friendly and easy to use. Reviews show that paper microfluidic devices are being used for in-field testing of pathogens and infection biomarkers [127,128]. A high-impact example showed paper-based microfluidics for DNA testing of malaria in low-resource rural settings in a first-in-human study [129]. Recent reviews also focus on paper-based chips for at-home nucleic acid testing and discuss design and validation challenges [130]. Even when the device is simple, quality control and validation are still needed so results remain reliable outside the lab [120,121].

3.3.4. Smartphone-Assisted and Portable Diagnostic Systems

Smartphone-assisted diagnostics use the phone as a reader, a power source, or a data and connectivity hub [119,131,133]. A classic example is a smartphone “dongle” that ran laboratory-style immunoassays at the point of care using phone power and optics [132]. Smartphones can also be combined with microfluidic devices to support portable testing with digital data handling [131,133]. A major benefit is that smartphones can store results, time-stamp them, and transmit them for remote review and surveillance [119,121]. Smartphone image analysis can also help read LFAs and improve accuracy for multianalyte rapid tests [134]. A scoping review found that user experience and workflow issues can affect adoption of mobile-linked diagnostics in community settings [135]. This means design should focus on clear instructions, privacy, and simple steps for non-expert users [121,135].

 doi:10.20944/preprints202603.1630.v1

3.4. Emerging Biosensor Technologies

Biosensors aim to detect pathogens or infection biomarkers quickly by turning a biological binding event into a measurable signal [136,137]. These systems are being developed to support faster testing, smaller devices, and more decentralized use [136,140].

3.4.1. Electrochemical Biosensors

Electrochemical biosensors measure electrical changes when a target binds on a sensor surface [137,138]. Common readouts include changes in current, voltage, or impedance [138,139].

These biosensors can be compact and can work with simple electronics, which supports point-of-care use [138,140]. In infectious diseases, electrochemical formats are used to detect pathogen antigens, nucleic acids, or host biomarkers [136,137,140]. Recent reviews describe key design choices, such as the biorecognition layer (antibodies, aptamers, or nucleic acids) and the electrode surface chemistry [138,140]. Electrochemical protein biosensors have been widely studied for viral infectious diseases and inflammatory biomarkers, and they often use signal amplification materials on the electrode [139]. A proof-of-concept study also showed an electrochemical biosensor for measuring sepsis-related biomarkers in an LPS-induced sepsis model [141]. Main challenges include non-specific binding, matrix interference from real samples, and the need for stable and reproducible sensor fabrication [136,138,140].

3.4.2. Optical and Plasmonic Biosensors

Optical biosensors measure changes in light signals caused by target binding or changes in the local environment near the sensor [137,142]. Surface plasmon resonance (SPR) is a common optical method that can detect binding events by measuring changes in refractive index near a metal surface. SPR and related plasmonic approaches can be used as label-free methods, but they often need more complex optics than strip tests [142,143]. Recent reviews describe progress in SPR platforms and assay designs that better handle complex biological samples [142].

Plasmonic biosensors include SPR and localized SPR, and they can also be combined with surface-enhanced fluorescence or Raman signals for higher sensitivity [145,147]. A review focused on plasmonic fluorescence sensors highlights their use for rapid detection in infectious disease settings [145]. Plasmonic approaches are also being developed for integrated point-of-care systems that combine sensing with other functions, such as sample handling and rapid heating steps [146].

Virus detection is a major application area for optical and plasmonic biosensors, including metamaterial-based approaches that aim for fast and sensitive readouts [147]. A recent example reported a disposable multiplexed plasmonic biosensor for identifying common respiratory viruses in nasopharyngeal swab samples [148]. A broader review on plasmonic biosensors discusses steps needed to move these platforms toward fully operative biomedical detection systems [144].

3.4.3. Nanomaterial-Based and Lab-on-a-Chip Systems

Nanomaterials are used in biosensors to improve sensitivity, speed, or signal amplification. Examples include metal nanoparticles, carbon materials, and other nano-structured surfaces that increase surface area and enhance signal generation [149,150]. A review on nano-modified biosensors highlights trends toward smart sensing, multiplex detection, and point-of-care testing for infectious diseases [149]. A systematic review on metal nanocluster-based biosensors summarizes optical and electrochemical formats for bacterial and viral detection [151]. Nanomaterials can also support miniaturized lab-on-a-chip devices by improving detection on small platforms and enabling integrated workflows [150,152]. Lab-on-a-chip systems aim to combine steps such as sample preparation, reaction, and detection in one small device. An editorial overview describes lab-on-a-chip as a practical approach for rapid microorganism identification, while also noting challenges in standardization and real-world deployment [152].

 doi:10.20944/preprints202603.1630.v1

3.5. Multi-Omics and Integrated Detection Approaches

Multi-omics means measuring more than one biological layer, such as RNA, proteins, and metabolites, to understand infection and improve detection [165,166]. In infectious diseases, multi-omics can support diagnosis, severity assessment, and treatment response monitoring by capturing both pathogen signals and host-response signals [15,166]. A main goal is to move from single markers to clinically useful signatures that are more accurate and more stable across patients [11,15].

3.5.1. Transcriptomics, Proteomics, and Metabolomics

Transcriptomics measures RNA expression patterns, often from blood, to reflect how the host responds to infection [153,154]. Host gene-expression signatures can help distinguish bacterial from viral infections in some settings [15,153,154]. Benchmarking studies show that many different gene signatures exist and performance can change across datasets, so robust validation is essential [17,154]. Some signatures work well for common infections but can have known limits for certain pathogen groups, such as intracellular bacteria [155]. Transcriptomic models have also been developed for sepsis severity and mortality risk, which links transcriptomics to clinical staging and prognosis [156,157]. Proteomics measures proteins, often using mass spectrometry, to detect pathogen components or host-response patterns [158,159].

Proteomics can support infectious disease diagnostics by profiling host proteins in body fluids and by measuring pathogen proteins when they are present [158,159]. Recent reviews describe proteomics as an emerging tool in clinical microbiology, but they also note challenges such as workflow complexity and standardization [158,160]. A recent study described untargeted proteomics that can identify human-pathogenic viruses directly from patient samples, which shows a “broad detection” concept similar to metagenomics [161]. Combining antibody to specific toxins can also increase the specificity of immune proteomics.

Metabolomics measures small molecules that change during infection due to pathogen metabolism, host metabolism, or both [14,162]. Metabolomics and VOC analysis can support noninvasive or rapid detection concepts, but these approaches still need strong clinical validation for routine use [23,162]. A review of infectious disease metabolomics describes how metabolite patterns can reflect diagnosis and also treatment or vaccine response [14,164].

A recent clinical-style study used metabolomics with machine learning to identify SARS-CoV-2 and other viruses directly from respiratory samples, which shows how metabolomics can be turned into an assay approach [163].

3.5.2. Multi-Analyte Integration for Clinically Useful Signatures

Multi-analyte integration combines multiple biomarkers into one signature or score to improve performance compared with single markers [11,15]. Integrated signatures can combine several host markers, several pathogen markers, or both together, depending on the clinical question [15,166].

Multi-marker signatures can be built from transcript panels, protein panels, or combined omics features [15,18–21]. Examples include host mRNA panels used to distinguish bacterial from viral infection patterns and support triage decisions [18,19]. Examples also include multi-protein panels, such as a combination of TRAIL, IP-10, and CRP used to support bacterial versus viral differentiation [20,21]. Multi-omics integration can also mean combining transcriptomics, proteomics, and metabolomics into one model. Reviews describe challenges in multi-omics integration, including batch effects, platform differences, missing data, and the risk of overfitting when many features are used [165,166]. Because of this, studies need careful design, external validation, and clear reporting to show that a signature is reliable and generalizable [154,165].

In infectious diseases, multi-omics reviews describe integration approaches for COVID-19 and for invasive fungal infections, which shows how integration is being applied across different pathogen types [167,168]. Overall, multi-omics and integrated detection approaches can improve diagnostic accuracy and may support more personalized infection management [15,166]. However,

 doi:10.20944/preprints202603.1630.v1

translation into routine care depends on simpler workflows, standardized pipelines, and evidence that the signature improves real clinical decisions [15,158,165].

4. Biomarkers in Infectious Diseases

4.1. Diagnostic Biomarkers

Diagnostic biomarkers are used to help decide if infection is present and what kind of infection it may be [11]. In practice, biomarkers are used together with symptoms, imaging, and microbiology tests.

4.1.1. Pathogen-Specific Biomarkers

Pathogen-specific biomarkers come from the pathogen or its products, so they give more direct evidence of infection. These should be unique to the specific pathogen usually tied to a conserved internal protein or a gene encoding a protein involved in virulence and pathogenicity. For invasive fungal infections, galactomannan and β-D-glucan are widely used non-culture biomarkers in selected patients [169,170] A meta-analysis showed that β-D-glucan has useful pooled accuracy for invasive fungal infection diagnosis, but it is not perfect [170]. A meta-analysis also showed that bronchoalveolar lavage galactomannan is useful for diagnosing invasive aspergillosis in the right clinical setting [171] These fungal biomarkers can still give false positives or false negatives, so interpretation must match the patient context and sample type [169–171] Cryptococcal antigen is a strong example of a pathogen antigen biomarker with high clinical value [10]. A multisite validation study supported good performance of cryptococcal antigen lateral flow testing for cryptococcal disease diagnosis [10]. Urinary antigen tests are another group of pathogen-specific biomarkers that can support rapid diagnosis. A systematic review and meta-analysis evaluated urine pneumococcal antigen testing for community-acquired pneumonia caused by Streptococcus pneumoniae [172]. In tuberculosis, urine lipoarabinomannan (LAM) is a pathogen biomarker that is useful in specific high-risk groups. WHO guidance supports LF-LAM use for diagnosing active TB in people living with HIV under defined conditions [173]. A recent prospective study measured LAM concentrations in urine and blood and evaluated real-world diagnostic accuracy in people with and without HIV [182] Newer studies and screening strategies continue to evaluate how LAM testing may improve case finding, including comparisons with FujiLAM in some settings [184].

4.1.2. Host Inflammatory Markers for Infection Detection

Host inflammatory markers rise when the body responds to infection, but they are not specific to one pathogen [174,181]. C-reactive protein (CRP) and procalcitonin (PCT) are two of the most used inflammatory biomarkers in clinical care [174]. A classic meta-analysis found that PCT had higher overall diagnostic accuracy than CRP for bacterial infection in hospitalized patients [175].

In pneumonia, a systematic review and meta-analysis showed that PCT alone has limited ability to separate viral from bacterial pneumonia [175]. In febrile infants, a meta-analysis compared PCT and CRP for predicting invasive or serious bacterial infection and reported different accuracy profiles across studies [176]. In febrile children more broadly, a systematic review found that CRP provides moderate help for ruling in or ruling out bacterial infection, but it cannot be used alone [181]. Because inflammation can also occur in noninfectious disease, these markers should be interpreted as supportive evidence, not proof of infection by themselves [174,179]. Inflammatory markers can also be affected by surgery and other sterile inflammatory states [183]. A meta-analysis in major gastrointestinal surgery showed that PCT and IL-6 have only moderate to good accuracy for postoperative infection or sepsis, with important study heterogeneity [183].

 doi:10.20944/preprints202603.1630.v1

4.1.3. Markers that Distinguish Infectious from Noninfectious Syndromes

Some biomarkers aim to distinguish infection-driven inflammation from noninfectious inflammation that looks similar. Host-response gene expression tests are one approach, because they measure a pattern of immune activation instead of one marker. SeptiCyte LAB is a host-response assay designed to help separate sepsis from noninfectious systemic inflammation in critically ill adults [19,177]. A large validation study reported diagnostic performance of SeptiCyte LAB for discriminating sepsis from SIRS in ICU patients [177]. A newer study validated SeptiCyte RAPID as a faster host-response test to discriminate sepsis from noninfectious inflammation [178]. Other multi-marker host-response tests focus on separating bacterial and viral infections, which supports early treatment decisions [18,20,21]. A 29-mRNA host-response test (InSep) was validated in emergency department patients to classify bacterial versus viral infection likelihood [18].

A protein signature using TRAIL, IP-10, and CRP has also been evaluated for bacterial versus viral respiratory infections [20]. A systematic review and meta-analysis summarized diagnostic test accuracy evidence for the TRAIL + IP-10 + CRP approach in febrile emergency department patients [21]. System-level evidence also compares many sepsis biomarkers and scores, showing that no single marker is best in every setting [180]. Guidelines for community-acquired pneumonia specifically advise that low PCT should not be used alone to withhold initial antibiotics when pneumonia is clinically suspected [179]. Overall, diagnostic biomarkers work best when they are targeted to the right patients and combined with clinical judgment and other tests [179,180].

4.2. Biomarkers for Disease Staging

Biomarkers for disease staging help clinicians describe how far an infection has progressed and how much risk the patient has [185]. Staging can be based on immune damage, pathogen burden, organ injury, or a combination of these [185,186].

4.2.1. Early Versus Advanced Disease

A clear staging example is HIV, where “advanced HIV disease” is defined by very low CD4 counts or severe HIV-related illness. These staging matters because advanced disease is linked with a higher risk of severe opportunistic infections and death. So, CD4-based staging helps decide who needs additional screening and preventive treatment [185]. Another common staging pattern is chronic infection with slow organ damage, where staging focuses on organ injury. In chronic hepatitis C, simple blood-based fibrosis scores can help separate early fibrosis from advanced fibrosis. This is useful because advanced fibrosis changes prognosis and care plans [186].

4.2.2. Localized Versus Disseminated Infection

A second staging question is whether infection is localized to one site or disseminated throughout the body. Disseminated infection often leads to higher levels of pathogen products in blood or urine because the organism is present in more places [187,188]. Urine lipoarabinomannan (LAM) detection in people living with HIV is linked to disseminated tuberculosis and advanced immunodeficiency. This helps explain why urine LAM testing performs best in severely ill or immunosuppressed patients [187]. Disseminated histoplasmosis is another example where antigen detection reflects disease burden and spread [188,189]. Evidence shows that urine antigen testing can be more sensitive than urine PCR for diagnosing disseminated histoplasmosis [189]. This supports antigen testing as a useful biomarker for disseminated disease in the right patient groups [188,193]. Antigen concentration can also help stage disease severity and dissemination in some infections. For cryptococcosis in HIV, higher blood cryptococcal antigen titers are associated with a higher chance of subclinical meningitis [192].

 doi:10.20944/preprints202603.1630.v1

4.2.3. Acute Versus Chronic Infection

A third staging question is whether infection is acute or chronic [190,191]. For hepatitis B, chronic infection is commonly defined by persistence of hepatitis B surface antigen over time, while acute infection is supported by markers like IgM anti-HBc in the right setting [190,191,194]. This serology pattern helps clinicians interpret whether the infection is new, ongoing, or past exposure [191,194].

4.3. Biomarkers of Therapeutic Response

Biomarkers of therapeutic response are markers that change during treatment and help show whether therapy is working [195]. Response biomarkers are most useful when they can be measured repeatedly and show a clear trend with improvement or worsening [195,196].

4.3.1. Dynamic Markers that Change During Treatment

Some response biomarkers are quantitative, so clinicians can follow the direction and speed of change over time [195]. In disseminated histoplasmosis, Histoplasma antigen levels usually decrease during effective therapy, and this decrease can be measured in urine or serum. This makes antigen kinetics a practical response marker when symptoms improve slowly or when clinical assessment is difficult [188,195]. In invasive aspergillosis, galactomannan can be used as a dynamic marker during antifungal treatment in selected patients [169,196]. A review supports galactomannan as a surrogate marker linked to outcome, especially when trends are followed over time [196]. Clinical data also show that early serum galactomannan trends during the first weeks of therapy can help predict later outcomes [197]. For some chronic viral infections, response monitoring uses pathogen nucleic acid levels as a dynamic marker [29,30]. In HIV, changes in plasma HIV-1 RNA during antiretroviral therapy are used to assess response and suppression [29]. In hepatitis C, on-treatment and post-treatment HCV RNA testing supports response assessment and later cure confirmation [30].

4.3.2. Predictors of Treatment Failure, Persistence, or Relapse

A strong response biomarker does not only change with improvement, but can also warn when a patient is at higher risk of failure or relapse. In tuberculosis, early microbiologic response is linked with later relapse risk across treatment regimens. A meta-regression model showed that month 2 culture status, together with treatment duration, predicts relapse risk at the regimen level [198]. This supports the idea that slower culture conversion can signal higher risk of persistence or relapse [38,198]. In disseminated histoplasmosis, antigen levels that fail to decline or that rise again can suggest persistent infection or relapse, although interpretation still needs clinical context [188,195]. In invasive aspergillosis, lack of improvement in galactomannan kinetics can also be a warning sign of poor response or worse outcomes [196,197].

4.3.3. Biomarkers that Support Antimicrobial Stewardship

Stewardship biomarkers help clinicians shorten antibiotic use when it is safe, which can reduce side effects and selection pressure for resistance [200]. Guidelines emphasize that biomarkers should support decisions but should not replace clinical assessment [199]. Procalcitonin-guided strategies have been evaluated across many studies in acute respiratory infections. A patient-level meta-analysis found that procalcitonin-guided care reduced antibiotic exposure and antibiotic-related side effects, with overall improved survival across settings [200]. In ICU settings, the PRORATA randomized trial showed that a procalcitonin-based algorithm reduced antibiotic exposure [201]. A patient-level meta-analysis of ICU trials also supports that procalcitonin-guided protocols can reduce antibiotic use without clear harm, although effects can vary by setting and protocol [203]. A large pragmatic trial in lower respiratory tract infection found that procalcitonin guidance did not substantially reduce antibiotic use compared with usual care, showing that implementation and clinician behavior matter [204]. In hospitalized patients with suspected sepsis, the ADAPT-Sepsis randomized trial found that a daily procalcitonin-guided protocol reduced total antibiotic duration

 doi:10.20944/preprints202603.1630.v1

compared with standard care with noninferior mortality [202]. C-reactive protein can also be used as a stewardship support marker in some settings [205,206]. In uncomplicated gram-negative bacteremia, a randomized trial found that CRP-guided antibiotic duration was noninferior to longer fixed-duration therapy [205]. A systematic review and meta-analysis also found that CRP-guided protocols can reduce antibiotic duration in hospitalized patients without clear harm, although the evidence base is still limited [206]. Overall, response and stewardship biomarkers work best when they are combined with clinical evaluation, source control assessment, and microbiology results [199,200].

4.4. Biomarkers of Cure

Biomarkers of cure aim to answer a simple question: is the infection truly cleared from the body [207]. This is harder than it sounds because patients can feel better before the pathogen is fully removed [208].

4.4.1. Distinguishing Microbiological Cure from Clinical Improvement

Microbiological cure means the pathogen is gone or no longer viable, based on microbiology evidence [31,207]. Clinical improvement means symptoms and signs get better, but this does not always prove that the pathogen is cleared [208]. Because of this, many trials and guidelines separate "clinical endpoints" from "microbiological endpoints." [207,208] In hepatitis C, cure is usually defined by a microbiology-based rule, which is sustained virologic response after treatment [30]. In tuberculosis programs, WHO uses outcome definitions that distinguish "cured" from "treatment completed," based on bacteriological evidence and reporting rules [31]. In some sexually transmitted infections, guidance uses "test-of-cure" only in selected situations, because routine testing can be misleading if done too early.

4.4.2. Challenges of Persistent Nucleic Acid or Antigen Detection

A major problem is that nucleic acid tests can stay positive after effective treatment because they may detect dead or non-viable pathogen DNA or RNA [209,213]. In tuberculosis, a prospective study showed false-positive Xpert MTB/RIF results in retested patients with prior TB, which highlights how DNA can persist without active disease [209]. Newer NAAT formats such as Xpert Ultra can improve sensitivity, but this can also increase detection of very low-level signals that may be hard to interpret in some contexts [210]. This "positive does not always mean active infection" issue also occurs with antigen tests. For malaria, HRP2-based rapid diagnostic tests can remain positive for weeks after treatment, so a positive test may reflect persistent antigen rather than live parasites [211]. The same timing problem is seen in bacterial STI test-of-cure [39,212]. For chlamydia, NAAT can remain positive for a short time after treatment, so testing too soon can misclassify cure as failure [212]. For viral infections, PCR positivity can last longer than infectiousness [213,214]. A systematic review and meta-analysis of coronaviruses described viral load and shedding patterns and how infectiousness does not always match PCR positivity over time [213]. This explains why persistently PCR-positive people may not always be contagious, depending on timing and clinical context [214].

4.4.3. Current Gaps in Validated Cure Biomarkers

A major problem is that nucleic acid tests can stay positive after effective treatment because they may detect dead or non-viable pathogen DNA or RNA [209,213]. In tuberculosis, a prospective study showed false-positive Xpert MTB/RIF results in retested patients with prior TB, which highlights how DNA can persist without active disease [209]. Newer NAAT formats such as Xpert Ultra can improve sensitivity, but this can also increase detection of very low-level signals that may be hard to interpret in some contexts [210]. This "positive does not always mean active infection" issue also occurs with antigen tests. For malaria, HRP2-based rapid diagnostic tests can remain positive for weeks after treatment, so a positive test may reflect persistent antigen rather than live parasites [211]. The same

 doi:10.20944/preprints202603.1630.v1

timing problem is seen in bacterial STI test-of-cure [39,212]. For chlamydia, NAAT can remain positive for a short time after treatment, so testing too soon can misclassify cure as failure [212]. For viral infections, PCR positivity can last longer than infectiousness [213,214]. A systematic review and meta-analysis of coronaviruses described viral load and shedding patterns and how infectiousness does not always match PCR positivity over time [213]. This explains why persistently PCR-positive people may not always be contagious, depending on timing and clinical context [214].

5. Disease-Specific Applications

5.1. Viral Infections

Viral diagnostic strategies depend strongly on the syndrome, the timing of symptoms, and the expected viral load in the sampled site [216,218]. In many viral infections, nucleic acid tests are most useful early, while serology becomes more useful later when antibodies have developed [218,225,228].

5.1.1. Respiratory Viral Infections

Respiratory viral infections often present with similar symptoms, so multiplex respiratory testing can improve speed and pathogen identification [83]. Influenza guidance emphasizes early testing when it will change treatment decisions, especially for high-risk patients [215,216]. CDC guidance summarizes influenza testing options, including rapid antigen tests and molecular tests, and explains that molecular tests are generally more sensitive [216]. For RSV, diagnostic testing choices depend on age, severity, and clinical setting, and CDC guidance outlines available methods and their typical use [217].

For COVID-19, IDSA provides guidance on molecular testing and how to interpret results across clinical scenarios [218]. Rapid antigen tests can be useful for quick decisions, but their sensitivity varies with viral load and timing [110]. Because rapid tests are commonly lateral flow assays, they support decentralized testing but still need careful interpretation in low viral load situations [108,110].

5.1.2. HIV, HBV, and HCV

For HIV, diagnosis commonly follows an algorithm that starts with a combination antigen/antibody immunoassay and then uses supplemental testing when needed. CDC and APHL provide a clear recommended laboratory HIV testing algorithm for serum or plasma specimens [219,220].

After diagnosis, viral load and CD4 count are key biomarkers used to monitor disease status and treatment response over time [29,221]. For hepatitis B, diagnosis and staging rely on serology patterns and, when needed, HBV DNA to assess viral replication and guide management [189,190,222].

CDC recommendations help interpret markers such as HBsAg and IgM anti-HBc to distinguish acute infection from chronic infection and prior exposure [190]. WHO guidance also supports chronic HBV care pathways that use these markers for diagnosis, monitoring, and treatment decisions [222]. For hepatitis C, diagnosis depends on anti-HCV testing followed by confirmatory HCV RNA testing to identify current infection. CDC guidance provides operational details for implementing HCV testing recommendations [223,224]. After treatment, sustained virologic response (SVR) is the standard virologic outcome used to define cure [30].

5.1.3. Arboviruses and Emerging Viral Diseases

Arbovirus testing is highly time-dependent because viremia is usually highest early, while IgM antibodies appear later [225,228,229]. For dengue, CDC guidance recommends using RT-PCR or NS1 antigen tests early in illness and serology later, depending on the day of symptoms [225]. A systematic review and meta-analysis compared common dengue reference tests and supports the

 doi:10.20944/preprints202603.1630.v1

timing-based use of RT-PCR, NS1 ELISA, and IgM ELISA [227]. Zika testing guidance emphasizes NAAT early and notes interpretation challenges for serology because of cross-reactivity with other flaviviruses [226,228]. CDC guidance also covers chikungunya and explains that RT-PCR is most useful early, while serology becomes useful later in illness [229]. A review of chikungunya laboratory diagnosis summarizes available assay types and how they are used [230]. West Nile virus diagnosis commonly uses serology, especially IgM, because viremia is often brief, and CDC guidance outlines the main testing approach [231]. Cross-reactivity between flaviviruses is a major problem for serology interpretation in dengue and Zika, and this needs careful clinical and epidemiologic context [226,232]. Emerging viral diseases require flexible testing strategies and clear public health reporting guidance. For Oropouche virus disease, CDC and PAHO provide updated recommendations for testing, reporting, and surveillance as outbreaks occur [233,234].

5.2. Bacterial Infections

Bacterial infections include acute life-threatening syndromes like sepsis, chronic infections like tuberculosis, and localized infections like many sexually transmitted infections [28,39,241]. Diagnostic choices depend on the clinical syndrome, specimen type, and whether rapid results will change treatment [237].

5.2.1. Sepsis and Bloodstream Infections

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection, and early recognition matters [28,199]. Bloodstream infection diagnosis still relies on blood cultures because they provide both organism identification and isolates for susceptibility testing. Guidance emphasizes correct blood culture collection, including timing, volume, and contamination prevention [235,236]. Diagnostic stewardship is important because unnecessary cultures and contaminated cultures can lead to false positives and inappropriate antibiotics [237]. Rapid molecular diagnostics can speed identification of pathogens and some resistance genes from positive blood cultures or directly from blood in selected platforms [238–240]. A systematic review and meta-analysis supports that some blood culture rapid identification panels have good diagnostic accuracy for many common bloodstream pathogens and resistance markers [239]. Another meta-analysis summarized performance of molecular tests for bloodstream infection diagnosis in real clinical settings [238]. A diagnostic accuracy study also reported performance of a direct-from-blood platform (T2Bacteria) for bloodstream infection diagnosis [240]. Evidence shows that rapid diagnostics for bloodstream infections can improve outcomes when combined with antimicrobial stewardship and clinical action [84]. So, the test is most useful when results reach the treating team quickly and lead to timely antibiotic adjustment [84,237].

5.2.2. Tuberculosis

Tuberculosis diagnosis today still uses a combination of clinical assessment, imaging, and microbiology testing, and rapid molecular tests are now central in many settings [116,241]. WHO consolidated TB guidelines recommend rapid molecular diagnostics for TB detection and for drug resistance testing in defined workflows [241]. Urine lipoarabinomannan (LF-LAM) is recommended by WHO for diagnosing active TB in people living with HIV under defined conditions, which supports diagnosis in very ill patients [173]. Drug-resistant TB diagnosis increasingly uses molecular tools that identify resistance-associated mutations [241]. A systematic review and meta-analysis evaluated targeted next-generation sequencing for diagnosing drug-resistant TB and supports its role as an advanced resistance testing approach [242]. These approaches can help guide therapy earlier than phenotypic testing in some settings, although implementation depends on laboratory capacity [241,242].

 doi:10.20944/preprints202603.1630.v1

5.2.3. Antimicrobial Resistance Detection

Sexually transmitted bacterial infections are often diagnosed using nucleic acid tests because they are sensitive and work well with noninvasive specimens [39]. Syphilis diagnosis relies on serologic testing algorithms, and CDC provides updated laboratory recommendations for interpreting and using syphilis tests [39,243]. For gonorrhea, NAAT is widely used for diagnosis, but antimicrobial resistance is a major concern [39,246]. CDC provides laboratory information and guidance relevant to gonorrhea testing and resistance concerns [244,246]. Targeted molecular assays can also help predict susceptibility for selected drugs, such as PCR assays that predict ciprofloxacin susceptibility using resistance markers [245].

5.3. Fungal Infections

5.3.1. Invasive Fungal Disease

Invasive fungal disease is a serious infection that can spread to blood and deep organs. It is most common in patients with weak immunity, after transplant, or in critical illness. Signs and imaging are often not specific, so diagnosis is not straightforward. Culture can take time and can be negative even when disease is present. Because of this, many clinical frameworks use a combined approach that includes host risk, imaging, and lab evidence [247]. Clinical guidelines describe how to diagnose and manage common invasive fungi such as Aspergillus, Candida, Cryptococcus, and Mucorales [259–262].

5.3.2. Antigen-Based, Molecular, and Host-Response Diagnostics

Antigen tests can support faster decisions than culture in many patients [247,248]. Galactomannan testing in serum or BAL is often used to support invasive aspergillosis diagnosis in high-risk patients [248,252,253]. 1,3-β-D-glucan is a broad fungal marker that can support detection of invasive fungal infection, but it does not identify the species and it can be affected by non-fungal factors [249,254]. Cryptococcal antigen lateral flow testing has strong performance in serum and CSF and is central for cryptococcosis diagnosis [250,255]. PCR tests can detect fungal DNA and can add value when culture is negative or delayed. Test performance depends on the sample type, assay design, and lab workflow, so results must be interpreted with the clinical context [248,256]. For candidemia, direct-from-blood panels such as T2Candida can detect common Candida species faster than blood culture in some settings [257]. For mucormycosis, serum Mucorales PCR has been evaluated in prospective studies and can support earlier recognition when tissue is hard to obtain [251,258]. Host-response methods are being studied as add-on tools, because direct fungal tests can miss early disease [247]. Most clinical guidance still relies mainly on clinical findings plus fungal tests, rather than host-response markers alone [258–262].

5.4. Parasitic and Neglected Tropical Diseases

5.4.1. Direct Detection Challenges

Direct detection works best when the parasite or its products are present in the sampled site at the time of testing. This can be hard when parasite levels are low, when shedding is intermittent, or when infection is in deep tissue [263]. For malaria, WHO recommends confirming suspected cases with microscopy or rapid diagnostic tests when possible [259]. Some malaria rapid tests depend on HRP2, and pfhrp2/pfhrp3 deletions can reduce test sensitivity and cause false-negative results [260,261]. For visceral leishmaniasis, rapid tests can support case finding, but performance varies by region and patient group, so local validation matters [262]. Molecular tests such as PCR can improve detection in some settings, but they may be limited by infrastructure and specimen access [267]. For schistosomiasis, egg microscopy can miss low-intensity infections, so antigen approaches are important in some settings [264,265].

 doi:10.20944/preprints202603.1630.v1

5.4.2. Serological Limitations

Antibody tests can support diagnosis for some parasitic infections, but antibodies can stay positive long after cure [270]. This makes it hard to separate past exposure from active infection using serology alone. Because of this, serology is often weak for cure assessment and treatment monitoring [270]. For chronic Chagas disease, diagnosis relies on serology, but serologic cure usually takes a long time after treatment [268,270].

5.4.2. Biomarkers for Staging and Cure

Biomarkers that reflect active infection are important for staging and for measuring cure in programs and trials [265,267]. For schistosomiasis, circulating antigen markers can track active infection better than antibodies in some settings [264,266]. A prospective cohort study showed that circulating antigen markers can fall after treatment even when antibodies remain positive [266]. Urine POC-CCA can be more sensitive than stool microscopy in many studies, but performance varies by setting and reference standard [265]. For Chagas disease, qPCR can measure parasite DNA and can show treatment effects earlier than standard serology in some studies [267]. Some antibody patterns are also being studied as surrogate markers of parasite persistence and response to therapy [269]. For human African trypanosomiasis, staging is important because treatment depends on disease stage [271]. Metabolomics studies have proposed candidate markers for diagnosis and staging, but these still need strong clinical validation for routine use [272].

6. Analytical and Clinical Validation of Assays and Biomarkers

6.1. Analytical Validation

Analytical validation shows whether an assay or biomarker test can detect and measure its target in a reliable way before it is used in patient care [284–289].

One key point is the limit of detection. This is the lowest amount of target that the test can detect with confidence. In infectious disease assays, the limit of detection should be checked in the real specimen type, because performance can change between blood, serum, plasma, urine, or cerebrospinal fluid [273,278]. The assay should also show good precision and reproducibility. This means the same sample should give similar results when it is tested many times in one run, on different days, and, when needed, at different sites. For quantitative assays, linearity should also be assessed so the signal changes in a predictable way across the measuring range [274,275].

Another important part is specificity. Infectious disease assays must detect the intended target without reacting with non-target organisms or host material. For this reason, validation should include studies of cross-reactivity with related pathogens and interference from substances such as blood components, drugs, transport media, or amplification inhibitors [276,278].

Preanalytical factors are also critical. Sample type, collection tube, transport temperature, delay before processing, freeze-thaw cycles, and storage conditions can all affect the final result. Studies have shown that pathogen nucleic acids and pathogen cell-free DNA may lose stability in some specimen types or under poor handling conditions, which can reduce sensitivity and increase variation [277,279,280].

Overall, analytical validation in infectious disease detection should cover the full testing pathway, from specimen collection to final readout. This is necessary to show that an assay or biomarker is sensitive, specific, stable, and reproducible in real clinical use [284–291].

6.2. Clinical Validation

Clinical validation shows how well an assay or biomarker works in real patients, not only in laboratory conditions [281]. It asks whether the test can correctly separate infected from noninfected patients and whether the result is useful for clinical decisions in the intended setting [281].

 doi:10.20944/preprints202603.1630.v1

The main measures are sensitivity and specificity. Sensitivity shows how well the test detects patients who truly have the infection. Specificity shows how well it excludes those who do not have the infection [281]. These measures are important for both pathogen-detection assays and host-response biomarkers, because missing true cases or creating false positives can both harm patient care [281].

Clinical validation should also report positive predictive value and negative predictive value. These measures are useful because they tell clinicians how likely a positive or negative result is to be correct in practice. However, predictive values change with disease prevalence, so the same test may perform differently in high-prevalence and low-prevalence settings [282].

Likelihood ratios are also valuable because they show how much a test result changes the probability of disease. A high positive likelihood ratio supports ruling in infection, while a low negative likelihood ratio supports ruling it out. These measures are often more clinically useful than sensitivity or specificity alone because they connect test results to bedside decision-making [283].

For continuous biomarkers, such as inflammatory proteins or multi-marker scores, ROC curves and area under the curve (AUC) are commonly used. The ROC curve shows the balance between sensitivity and specificity across different thresholds. The AUC gives a summary of overall discrimination, while the selected clinical cutoff determines the final tradeoff between missed cases and false positives [284]. Because of this, cutoffs should be chosen according to the intended clinical purpose, such as screening, triage, confirmation, or treatment monitoring [281,284].

A major challenge in infectious disease diagnostics is the choice of the reference standard. Many studies compare a new assay with a so-called gold standard, but the available standard is often imperfect [281,285,286]. This is especially important when a new molecular assay or biomarker may be more sensitive than older culture-based or serological methods [285].

When the reference standard is imperfect, the measured sensitivity and specificity of the new test can be biased. In some cases, the new test may appear falsely inaccurate because the comparator misses true infections. This problem has been shown clearly in infectious disease studies, including work on leptospirosis, where use of an imperfect standard changed the estimated performance of newer diagnostic tests [286].

For this reason, clinical validation should use the best available comparator and should clearly explain its limits. In difficult settings, researchers may need composite reference standards, expert clinical adjudication, or latent class approaches to better estimate the true performance of an assay or biomarker [285,286]. This is essential if new infectious disease diagnostics are to move from promising technology to reliable clinical tools [281,285].

6.3. Study Design Considerations

Study design strongly affects how well a new assay or biomarker appears to perform. Prospective studies are usually better because they test patients as they appear in real practice. In contrast, case-control designs often compare clear positive cases with clear negative controls, which can make the test look better than it really is [287]. A key problem is spectrum bias. This happens when the study does not include the full range of patients seen in clinical care, such as mild disease, early infection, mixed syndromes, or patients with related conditions. For infectious disease diagnostics, this can give accuracy estimates that are too high and less useful for real-world use [287,288].

Studies also need enough patients to give stable results. Small studies may produce wide confidence intervals and weak estimates of sensitivity and specificity. For this reason, sample size and statistical power should be considered early in study planning [289].

Finally, validation should use clinically meaningful sample sets. The tested samples should match the intended patient group, specimen type, and care setting. This is important if new infectious disease assays and biomarkers are meant to support real clinical decisions [288].

 doi:10.20944/preprints202603.1630.v1

6.4. Reporting and Evidence Synthesis Standards

Clear reporting is essential so readers can judge whether a diagnostic study is valid and clinically useful. STARD gives a structured checklist for reporting diagnostic accuracy studies, including patient selection, index test methods, reference standard, and analysis [26].

For reviews, PRISMA 2020 helps authors report systematic reviews in a clear and complete way. When the review focuses on diagnostic test accuracy, PRISMA-DTA is especially useful because it is designed for studies of sensitivity, specificity, and related measures [290,291].

Using these standards improves transparency, makes studies easier to compare, and helps build stronger evidence for new infectious disease assays and biomarkers [26,290,291].

7. Translational and Implementation Challenges

7.1. From Biomarker Discovery to Clinical Adoption

Many infectious disease biomarkers look promising in early studies, but only a small number become routine clinical tests [16,292]. Results are often hard to reproduce across different patient groups, specimen types, study sites, and testing methods, and strong external validation is still missing for many candidates. Without this step, a marker may perform well in discovery work but fail in real clinical use.

Another major problem is the lack of standardization across platforms. Different studies may use different sample handling methods, analytical platforms, normalization steps, thresholds, and result formats, which makes comparison difficult. This is especially important for host-response and multi-marker assays, where small technical differences can change the final result [16,292].

7.2. Practical Deployment Issues

Even when an assay has good laboratory performance, it may still be hard to use in practice [119,293,294]. Turnaround time must be short enough to affect treatment, infection control, or referral decisions, and the cost must be realistic for the health system where the test will be used. Tests that need complex preparation, many manual steps, or special expertise may not fit busy clinical workflows [293,294].

Infrastructure is another key issue [119,293,294]. Centralized laboratories can support complex instruments, trained staff, quality systems, and digital reporting, but these resources are not always available outside major centers [293,294]. In low-resource settings, diagnostics often need to be simple, robust, affordable, and usable with limited electricity, limited cold chain, and limited laboratory support [119,293]. For infectious diseases, implementation success depends not only on the assay itself, but also on whether the full testing pathway can work in the intended setting [119,293,294].

7.3. Regulatory and Quality-Assurance Considerations

Analytical novelty alone is not enough for adoption [292,295]. A new assay or biomarker may be scientifically interesting, but regulators and health systems also need evidence that it supports its intended clinical use and adds value compared with current care. In other words, strong analytical performance must be matched by clear clinical performance and meaningful clinical utility [292,295].

Standardization and harmonization are also important after a test enters practice. Results should be as consistent as possible across sites, operators, and time periods, which requires clear procedures, quality controls, and comparable reporting systems [295,296]. Post-implementation quality monitoring is also essential and should include complaint review, adverse event reporting, corrective action, and continuing surveillance of test performance in real use [296].

 doi:10.20944/preprints202603.1630.v1

7.4. Equity and Global Access

Access to good diagnostics is still uneven across the world [263,294,297]. Many endemic and neglected infections affect populations with the weakest laboratory systems, so the places with the greatest need often have the least access to reliable testing. This gap can delay diagnosis, support empirical treatment, and weaken disease control efforts [263,294].

Point-of-care access is especially important in resource-limited settings. For these settings, infectious disease diagnostics should not only be accurate, but also affordable, rapid, easy to use, and linked to treatment pathways and supply systems [119,263,293]. Wider access will require better alignment between diagnostic design, health-system needs, and national planning for essential in vitro diagnostics [263,297].Point-of-care accessand Over the Counter Self tests in resource-limited settings.

8. Future Directions

8.1. Future Directions

Future infectious disease diagnostics will likely move from single markers toward patient-specific and context-specific biomarker signatures. In practice, the same assay may need different interpretation depending on age, immune status, syndrome, sample type, and stage of disease. Host-response and multi-marker approaches are already moving in this direction, but future work must make these signatures faster, simpler, and more reliable in real patient populations [15,298].

8.2. Artificial Intelligence and Computational Integration

Artificial intelligence will likely become more important in the interpretation of complex infectious disease data. Machine-learning models can combine several markers at the same time, such as transcriptomic, proteomic, metabolomic, and clinical variables, and turn them into one clinically useful output. Digital decision-support tools may also help decentralized sites use advanced tests more safely, but this will require good training data, external validation, interoperability, and careful control of bias [299,300]. Indeed, combining biomarkers with pathogen specific biomarker greatly enhances specificity mathematically. Mock example, a CRP elevation test sand a specific antibody: Test 1 Specificity: 95% (false positive rate); Test 2 Specificity: 90% (false positive rate) Combined Specificity = 1-(0.05 X 0.10)=1-0.005=99.5% For some diseases with a need for better tests this may be the most useful approach.

8.3. Home-Based and Decentralized Testing

Home-based and decentralized testing will likely expand beyond simple self-tests. Future systems may combine self-testing, connected devices, smartphone guidance, remote result sharing, telemedicine review, and rapid linkage to treatment. For infectious diseases, the main goal is not only to give a result at home, but also to connect that result quickly to the next step in care [119,130,301].

8.4. Next-Generation Biomarkers

Several next-generation biomarker classes are especially promising. Host transcriptomic signatures may improve separation of bacterial, viral, and noninfectious syndromes. Cell-free nucleic acids and extracellular vesicles may offer less invasive ways to detect infection and follow biological change over time. Longitudinal immune profiling may also become more useful because repeated measurements can show disease evolution and treatment response more clearly than a single time point [15,17,298,302,303].

 doi:10.20944/preprints202603.1630.v1

8.5. Biomarkers of Cure and Relapse Prediction

Biomarkers of cure and relapse prediction remain one of the most important unmet needs in the field. This is especially clear in tuberculosis, where better markers are still needed to show true pathogen clearance, monitor treatment response, and identify patients at risk of later relapse. Future translational research should prioritize biomarkers that change during therapy, can be measured repeatedly, and remain reliable across different populations and settings [214,303,304].

9. Conclusions

This review shows that infectious disease diagnostics are no longer limited to proving that a pathogen is present. This is increasingly important to distinguish between active infection, carriers and asymptomatic patients. Active infections typically show symptoms and shed organism according to their respective pathogenicity. Carriers do not show symptoms and shed organisms longer term in general. For example, Streptococcus pyogenes in the oral cavity. Asymptomatic run through the generally normal time course of infection and shed organism. However they do not have symptoms. For example, SARS-CoV2. In particular with molecular testing, many organisms are found on healthy humans and would exhibit a positive PCR test (for example see Table 1). Indeed, the ability of diagnostic tests to detect microbial material without always proving active disease stresses the importance of combined clinical symptomology with diagnostic results.

New assays and biomarkers are increasingly being developed to answer wider clinical questions, including what is causing illness, how severe it is, how disease stage should be understood, whether treatment is working, and whether cure has truly been achieved. At the same time, the review makes clear that better analytical performance does not automatically lead to better patient care. Results must still be interpreted carefully in the setting of colonization, contamination, residual microbial signals, host variability, and imperfect reference standards. For this reason, the real value of any new diagnostic tool depends on clinical usefulness, not novelty alone. Progress in the field will require strong validation in real patient populations, better standardization across platforms, practical workflows, quality assurance, and wider access across different health systems. Looking ahead, the most promising advances are likely to come from integrated pathogen and host-response approaches, more effective decentralized testing, and reliable biomarkers for treatment response, cure, and relapse prediction. Overall, the field will move forward most effectively when innovation is matched by interpretability, clinical relevance, and equitable implementation.

Author Contributions: These authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Walker DH. Principles of Diagnosis of Infectious Diseases. Pathobiology of Human Disease. 2014;222–225. doi:10.1016/B978-0-12-386456-7.01713-5. (PMCID: PMC7149376).
2. Washington JA. Chapter 10: Principles of Diagnosis. In: Baron S, ed. Medical Microbiology. 4th ed. University of Texas Medical Branch at Galveston; 1996. (NCBI Bookshelf: NBK8014).
3. Apfalter P, et al. In-house nucleic acid amplification assays in research: how much quality control is needed before one can rely upon the results? J Clin Microbiol. 2005;43(12):5835–5841. doi:10.1128/JCM.43.12.5835-5841.2005. (PMID: 16333064; PMCID: PMC1317225).
4. Vainionpää R, Leinikki P. Diagnostic Techniques: Serological and Molecular Approaches. Encyclopedia of Virology. 2008;29–37. doi:10.1016/B978-012374410-4.00585-9. (PMCID: PMC7150332).

 doi:10.20944/preprints202603.1630.v1

5. Atallah JAJ, Mansour MK. Implications of Using Host Response-Based Molecular Diagnostics on the Management of Bacterial and Viral Infections: A Review. Front Med (Lausanne). 2022. doi:10.3389/fmed.2022.805107.
6. Woodhouse EW, McClain MT, Woods CW. Harnessing the host response for precision infectious disease diagnosis. Clin Microbiol Rev. 2024;37(4):e00078-24. doi:10.1128/cmr.00078-24. (PMID: 39404266; PMCID: PMC11629621).
7. McDonald LC, Gerding DN, Johnson S, et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by IDSA and SHEA. Clin Infect Dis. 2018;66(7):e1–e48. doi:10.1093/cid/cix1085. (PMID: 29462280; PMCID: PMC6018983).
8. Drancourt M, Michel-Lepage A, Boyer S, Raoult D. The point-of-care laboratory in clinical microbiology. Clin Microbiol Rev. 2016;29:429–447. doi:10.1128/CMR.00090-15. (PMID: 27029593; PMCID: PMC4861988).
9. Sethi S, Nanda R, Chakraborty T. Clinical application of volatile organic compound analysis for detecting infectious diseases. Clin Microbiol Rev. 2013;26(3):462–475. doi:10.1128/CMR.00020-13. (PMID: 23824368; PMCID: PMC3719490).
10. Boulware DR, Rolfes MA, Rajasingham R, et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerg Infect Dis. 2014;20(1):45–53. doi:10.3201/eid2001.130906. (PMID: 24378231; PMCID: PMC3884728)
11. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource. NCBI Bookshelf.
12. Tucci P, González-Sapienza G, Marín M. Pathogen-derived biomarkers for active tuberculosis diagnosis. Frontiers in Microbiology. 2014. doi:10.3389/fmicb.2014.00549.
13. Lass-Flörl C, Samardzic E, Knoll M. Serology anno 2021—fungal infections: from invasive to chronic. Clinical Microbiology and Infection. 2021;27(9):1230–1241. doi:10.1016/j.cmi.2021.02.005.
14. Rahman M, Schellhorn HE. Metabolomics of infectious diseases in the era of personalized medicine. Frontiers in Molecular Biosciences. 2023;10:1120376. doi:10.3389/fmolb.2023.1120376.
15. Woodhouse EW, McClain MT, Woods CW. Harnessing the host response for precision infectious disease diagnosis. Clinical Microbiology Reviews. 2024. doi:10.1128/cmr.00078-24.
16. Atallah JAJ, Mansour MK. Implications of Using Host Response-Based Molecular Diagnostics on the Management of Bacterial and Viral Infections: A Review. Frontiers in Medicine. 2022. doi:10.3389/fmed.2022.805107.
17. Bodkin N, Ross M, McClain MT, et al. Systematic comparison of published host gene expression signatures for bacterial/viral discrimination. Genome Medicine. 2022;14:18. doi:10.1186/s13073-022-01025-x.
18. Safarika A, et al. A 29-mRNA host response test from blood accurately distinguishes bacterial and viral infections among emergency department patients. Intensive Care Medicine Experimental. 2021;9(1):31. doi:10.1186/s40635-021-00394-8.
19. McHugh L, Seldon TA, Brandon RA, et al. A Molecular Host Response Assay to Discriminate Between Sepsis and Infection-Negative Systemic Inflammation in Critically Ill Patients. PLOS Medicine. 2015. doi:10.1371/journal.pmed.1001916.
20. Halabi S, Shiber S, Paz M, et al. Host test based on TRAIL, IP-10 and CRP for differentiating bacterial and viral respiratory tract infections in adults: diagnostic accuracy study. Clinical Microbiology and Infection. 2023;29(9):1159–1165. doi:10.1016/j.cmi.2023.05.033.
21. Kandula M, Farrell JJ. Diagnostic test accuracy of TRAIL, IP-10 and CRP biomarker combination (MeMed BV test) for discrimination of viral vs bacterial infections among ED patients with febrile illness: a systematic review and meta-analysis. Open Forum Infectious Diseases. 2023;10(Suppl 2):ofad500.912. doi:10.1093/ofid/ofad500.912.
22. O'Neal HR Jr, Sheybani R, Kraus CK, et al. Cellular host response sepsis test for risk stratification of patients in the emergency department: a pooled analysis. Academic Emergency Medicine. 2024;31(9):883–893. doi:10.1111/acem.14923.
23. Sethi S, Nanda R, Chakraborty T. Clinical application of volatile organic compound analysis for detecting infectious diseases. Clinical Microbiology Reviews. 2013;26(3):462–475. doi:10.1128/CMR.00020-13.

 doi:10.20944/preprints202603.1630.v1

24. Caliendo AM, Gilbert DN, Ginocchio CC, Hanson KE, May L, Quinn TC, et al. Better tests, better care: improved diagnostics for infectious diseases. Clin Infect Dis. 2013;57(Suppl 3):S139–S170. doi:10.1093/cid/cit578.
25. Sullivan KV. Diagnostic Stewardship in Clinical Microbiology, Essential Partner to Antimicrobial Stewardship. Clin Chem. 2022;68(1):75–82. doi:10.1093/clinchem/hvab206.
26. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies. Radiology. 2015;277(3):826–832. doi:10.1148/radiol.2015151516.
27. Bard JD, McElvania E. Panels and Syndromic Testing in Clinical Microbiology. Clin Lab Med. 2020;40(4):393–420. doi:10.1016/j.cll.2020.08.001.
28. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801–810. doi:10.1001/jama.2016.0287.
29. Panel on Antiretroviral Guidelines for Adults and Adolescents. Plasma HIV-1 RNA (Viral Load) and CD4 Count Monitoring. ClinicalInfo.HIV.gov. Updated Sep 25, 2025. https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/plasma-hiv-1-rna-cd4-monitoring
30. AASLD/IDSA HCV Guidance Panel. Monitoring Patients Who Are Starting HCV Treatment, Are on Treatment, or Have Completed Therapy. Updated Dec 19, 2023. https://www.hcvguidelines.org/guidance/monitoring-patients-who-are-starting-hcv-treatment-are-on-treatment-or-have-completed-therapy/
31. World Health Organization. Definitions and reporting framework for tuberculosis – 2013 revision. WHO; 2013. https://iris.who.int/bitstream/handle/10665/79199/9789241505345_eng.pdf
32. Markussen DL, et al. Diagnostic Stewardship in Community-Acquired Pneumonia With Syndromic Molecular Testing: A Randomized Clinical Trial. JAMA Netw Open. 2024;7(3):e240830. doi:10.1001/jamanetworkopen.2024.0830.
33. Candel FJ, et al. The role of rapid multiplex molecular syndromic panels in the clinical management of infections in critically ill patients: an experts-opinion document. Crit Care. 2024;28(1):440. doi:10.1186/s13054-024-05224-3.
34. Wang Y, Chen T, Zhang S, et al. Clinical evaluation of metagenomic next-generation sequencing in unbiased pathogen diagnosis of urinary tract infection. J Transl Med. 2023;21:762. doi:10.1186/s12967-023-04562-0.
35. World Health Organization. Outcome definitions (TB Knowledge Sharing Platform). Accessed Feb 26, 2026. https://tbksp.who.int/en/node/3027
36. World Health Organization. WHO consolidated guidelines on tuberculosis (monitoring and follow-up sections for DS-TB and DR-TB). NCBI Bookshelf (online version). https://www.ncbi.nlm.nih.gov/books/NBK588562/
37. Babu S. Biomarkers for Treatment Monitoring in Tuberculosis: A New Hope. EBioMedicine. 2017;26:13–14. doi:10.1016/j.ebiom.2017.11.002.
38. Lu P, Liu Q, Martinez L, et al. Time to sputum culture conversion and treatment outcome of patients with multidrug-resistant tuberculosis: a prospective cohort study from urban China. Eur Respir J. 2017;49(3):1601558. doi:10.1183/13993003.01558-2016.
39. Workowski KA, Bachmann LH, Chan PA, et al. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep. 2021;70(4):1–187. https://www.cdc.gov/mmwr/volumes/70/rr/rr7004a1.htm
40. Centers for Disease Control and Prevention. Clinical Care of Hepatitis C. Updated Jan 31, 2025. https://www.cdc.gov/hepatitis-c/hcp/clinical-care/index.html
41. Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev. 2006;19(1):165–256. doi:10.1128/CMR.19.1.165-256.2006.
42. Mackay IM. Real-time PCR in the microbiology laboratory. Clin Microbiol Infect. 2004;10(3):190–212. doi:10.1111/j.1198-743X.2004.00722.x.
43. Engstrom-Melnyk J, Rodríguez PL, Peraud O. Clinical applications of quantitative real-time PCR in virology. Methods Mol Biol. 2015. (Full text available via PMC: PMC7148891).

 doi:10.20944/preprints202603.1630.v1

44. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611–622. doi:10.1373/clinchem.2008.112797.
45. Johnson G, Nour AA, Nolan T, Huggett JF, Bustin SA. Minimum information necessary for quantitative real-time PCR experiments. Methods Mol Biol. 2014;1160:5–17. doi:10.1007/978-1-4939-0733-5_2.
46. Clinical and Laboratory Standards Institute (CLSI). Molecular Diagnostic Methods for Infectious Diseases. CLSI guideline MM03, 3rd ed. Wayne, PA: CLSI; 2015.
47. Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE. Multiplex PCR: optimization and application in diagnostic virology. Clin Microbiol Rev. 2000;13(4):559–570. doi:10.1128/CMR.13.4.559-570.2000.
48. Clinical and Laboratory Standards Institute (CLSI). Validation and Verification of Multiplex Nucleic Acid Assays. CLSI guideline MM17, 2nd ed. Wayne, PA: CLSI; 2018.
49. Huang HS, Tsai CL, Chang J, et al. Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis. Clin Microbiol Infect. 2018;24(10):1055–1063. doi:10.1016/j.cmi.2017.11.018.
50. Sanghavi SK, Bullotta A, Husain S, Rinaldo CR. Clinical evaluation of multiplex real-time PCR panels for rapid detection of respiratory viral infections. J Med Virol. 2012;84(1):162–169. doi:10.1002/jmv.22186.
51. Clark TW, Lindsley K, Wigmosta TB, et al. Rapid multiplex PCR for respiratory viruses reduces time to result and improves clinical care: results of a systematic review and meta-analysis. J Infect. 2023;86(5):462–475. doi:10.1016/j.jinf.2023.03.005.
52. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci U S A. 1999;96(16):9236–9241. doi:10.1073/pnas.96.16.9236.
53. Huggett JF, et al. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clin Chem. 2013;59(6):892–902. doi:10.1373/clinchem.2013.206375.
54. Huggett JF, et al. The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. Clin Chem. 2020;66(8):1012–1029. doi:10.1093/clinchem/hvaa125.
55. Salipante SJ, Jerome KR. Digital PCR—An Emerging Technology with Broad Applications in Microbiology. Clin Chem. 2020;66(1):117–123. doi:10.1373/clinchem.2019.304048.
56. Dominguez LS, et al. Present and Future Applications of Digital PCR in Infectious Diseases Diagnosis. Diagnostics (Basel). 2024;14(9):931. doi:10.3390/diagnostics14090931.
57. Morley AA. Digital PCR: A brief history. Biomol Detect Quantif. 2014;1(1):1–2. doi:10.1016/j.bdq.2014.06.001.
58. Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28(12):E63. doi:10.1093/nar/28.12.e63.
59. Mori Y, Kanda H, Notomi T. Loop-mediated isothermal amplification (LAMP): recent progress in research and development. J Infect Chemother. 2013;19(3):404–411. doi:10.1007/s10156-013-0590-0.
60. World Health Organization. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. WHO; 2016. (ISBN: 978-92-4-151118-6).
61. Shete PB, Farr K, Strnad L, Gray CM, Cattamanchi A. Diagnostic accuracy of TB-LAMP for pulmonary tuberculosis: a systematic review and meta-analysis. BMC Infect Dis. 2019;19(1):268. doi:10.1186/s12879-019-3881-y.
62. Kidd SP, et al. Reverse-transcription loop-mediated isothermal amplification has high accuracy for detecting SARS-CoV-2 in saliva and swabs in multisite clinical evaluation. J Mol Diagn. 2022. doi:10.1016/j.jmoldx.2021.12.007.
63. Piepenburg O, Williams CH, Stemple DL, Armes NA. DNA detection using recombination proteins (RPA). PLoS Biol. 2006;4(7):e204. doi:10.1371/journal.pbio.0040204.
64. Lobato IM, O'Sullivan CK. Recombinase polymerase amplification: Basics, applications and recent advances. TrAC Trends Anal Chem. 2018;98:19–35. doi:10.1016/j.trac.2017.10.015.
65. Mota DS, et al. Recombinase polymerase amplification in the molecular diagnosis of microbiological targets and its applications. Can J Microbiol. 2022. doi:10.1139/cjm-2021-0329.

 doi:10.20944/preprints202603.1630.v1

66. Zhang S, Duan M, Li S, et al. Current status of recombinase polymerase amplification technologies for the detection of pathogenic microorganisms. Diagn Microbiol Infect Dis. 2023;108(1):116097. doi:10.1016/j.diagmicrobio.2023.116097.
67. Hueso L, Martorell S, Sena-Torralba A, et al. Recombinase polymerase amplification technology for point-of-care diagnosis of neglected tropical diseases. Int J Infect Dis. 2025;153:107831. doi:10.1016/j.ijid.2025.107831.
68. Gootenberg JS, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438–442. doi:10.1126/science.aam9321.
69. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436–439. doi:10.1126/science.aar6245.
70. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018;360(6387):439–444. doi:10.1126/science.aaq0179.
71. Myhrvold C, et al. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444–448. doi:10.1126/science.aas8836.
72. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019;14(10):2986–3012. doi:10.1038/s41596-019-0210-2.
73. Kaminski MM, Abudayyeh OO, Gootenberg JS, Zhang F, Collins JJ. CRISPR-based diagnostics. Nat Biomed Eng. 2021;5(7):643–656. doi:10.1038/s41551-021-00760-7.
74. Ghouneimy A, Mahas A, Marsic T, Aman R, Mahfouz M. CRISPR-Based Diagnostics: Challenges and Potential Solutions toward Point-of-Care Applications. ACS Synth Biol. 2023;12(1):1–16. doi:10.1021/acssynbio.2c00496.
75. Broughton JP, et al. CRISPR–Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020;38(7):870–874. doi:10.1038/s41587-020-0513-4.
76. Patchsung M, et al. Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. Nat Biomed Eng. 2020;4(12):1140–1149. doi:10.1038/s41551-020-00603-x.
77. Joung J, et al. Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. N Engl J Med. 2020;383(15):1492–1494. doi:10.1056/NEJMc2026172.
78. Wang Z, Wang Q, Zhang J, et al. CRISPR-driven diagnostics: Molecular mechanisms, clinical efficacy and translational challenges. Clin Transl Med. 2025;15(10):e70482. doi:10.1002/ctm2.70482.
79. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev. 2018;31(1):e00024-17. doi:10.1128/CMR.00024-17.
80. Dien Bard J, McElvania E. Panels and syndromic testing in clinical microbiology. Clin Lab Med. 2020;40(4):393–420. doi:10.1016/j.cll.2020.08.001.
81. Subedi S, Harris PNA, Hall L, Paterson DL. Practical considerations for implementation of syndromic panel and diagnostic stewardship in the era of syndromic panel testing. Clin Microbiol Infect. 2025;31(11):1822–1827. doi:10.1016/j.cmi.2025.06.013.
82. Dumm RE, Marlowe EM, Patterson L, et al. The foundation for the microbiology laboratory's essential role in diagnostic stewardship: an ASM Laboratory Practices Subcommittee report. J Clin Microbiol. 2024;62(10):e00960-24. doi:10.1128/jcm.00960-24.
83. Clark TW, Lindsley K, Wigmosta TB, et al. Rapid multiplex PCR for respiratory viruses reduces time to result and improves clinical care: results of a systematic review and meta-analysis. J Infect. 2023;86(5):462–475. doi:10.1016/j.jinf.2023.03.005.
84. Timbrook TT, Morton JB, McConeghy KW, et al. The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis. Clin Infect Dis. 2017;64(1):15–23. doi:10.1093/cid/ciw649.
85. Axelrad JE, Freedberg DE, Whittier S, Greendyke W, Lebwohl B, Green DA. Impact of Gastrointestinal Panel Implementation on Health Care Utilization and Outcomes. J Clin Microbiol. 2019;57(3):e01775-18. doi:10.1128/JCM.01775-18.

 doi:10.20944/preprints202603.1630.v1

86. Trujillo-Gómez J, Tsokani S, Arango-Ferreira C, et al. Biofire FilmArray Meningitis/Encephalitis panel for the aetiological diagnosis of central nervous system infections: a systematic review and diagnostic test accuracy meta-analysis. EClinicalMedicine. 2022;44:101275. doi:10.1016/j.eclinm.2022.101275.
87. Saif NT, Coffey KC, et al. Clinical decision support for gastrointestinal panel testing. Antimicrob Stewardsh Healthc Epidemiol. 2024;4(1):e22. doi:10.1017/ash.2024.15.
88. Chiu CY, Miller SA. Clinical metagenomics. Nat Rev Genet. 2019;20(6):341–355. doi:10.1038/s41576-019-0113-7.
89. Gu W, Miller S, Chiu CY. Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. Annu Rev Pathol. 2019;14:319–338. doi:10.1146/annurev-pathmechdis-012418-012751.
90. Wilson MR, Sample HA, Zorn KC, et al. Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis. N Engl J Med. 2019;380(24):2327–2340. doi:10.1056/NEJMoa1803396.
91. Wilson MR, Naccache SN, Samayoa E, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med. 2014;370(25):2408–2417. doi:10.1056/NEJMoa1401268.
92. Schlaberg R, Chiu CY, Miller S, Procop GW, Weinstock G, et al. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection. Arch Pathol Lab Med. 2017;141(6):776–786. doi:10.5858/arpa.2016-0539-RA.
93. Roy S, Coldren C, Karunamurthy A, et al. Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines: A Joint Recommendation of the Association for Molecular Pathology and the College of American Pathologists. J Mol Diagn. 2018;20(1):4–27. doi:10.1016/j.jmoldx.2017.11.003.
94. Gaston DC. Clinical Metagenomics for Infectious Diseases: Progress toward Operational Value. J Clin Microbiol. 2023;61(2):e01267-22. doi:10.1128/jcm.01267-22.
95. Tan JK, Servellita V, Stryke D, et al. Laboratory validation of a clinical metagenomic next-generation sequencing assay for respiratory virus detection and discovery. Nat Commun. 2024;15:9016. doi:10.1038/s41467-024-51470-y.
96. Alcolea-Medina A, Snell LB, Humayun G, et al. Rapid pan-microbial metagenomics for pathogen detection and personalised therapy in the intensive care unit: a single-centre prospective observational study. Lancet Microbe. 2025;6(10):101174. doi:10.1016/j.lanmic.2025.101174.
97. Strong MJ, Xu G, Morici L, et al. Microbial contamination in next generation sequencing: implications for sequence-based analysis of clinical samples. PLoS Pathog. 2014;10(11):e1004437. doi:10.1371/journal.ppat.1004437.
98. Hasan MR, Rawat A, Tang P, et al. Depletion of human DNA in spiked clinical specimens to improve the sensitivity of pathogen detection by next generation sequencing. J Clin Microbiol. 2016;54(4):919–927. doi:10.1128/JCM.03050-15.
99. Deurenberg RH, Bathoorn E, Chlebowicz MA, et al. Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol. 2017;243:16–24. doi:10.1016/j.jbiotec.2016.12.022.
100. Wheeler NE, Price V, Cunningham-Oakes E, et al. Innovations in genomic antimicrobial resistance surveillance. Lancet Microbe. 2023;4(12):e1063–e1070. doi:10.1016/S2666-5247(23)00285-9.
101. Salipante SJ, Sengupta DJ, Rosenthal C, et al. Rapid 16S rRNA Next-Generation Sequencing of Polymicrobial Clinical Samples for Diagnosis of Complex Bacterial Infections. PLoS One. 2013;8(5):e65226. doi:10.1371/journal.pone.0065226.
102. Cummings LA, Kurosawa K, Hoogestraat DR, et al. Clinical Next Generation Sequencing Outperforms Standard Microbiological Culture for Characterizing Polymicrobial Samples. Clin Chem. 2016;62(11):1465–1473. doi:10.1373/clinchem.2016.258806.
103. Yusuf E, Allerberger F. Infectious disease serology in 2021. Clin Microbiol Infect. 2021;27(9):1204–1206. doi:10.1016/j.cmi.2021.06.020.
104. Vainionpää R, Leinikki P. Diagnostic Techniques: Serological and Molecular Approaches. Encyclopedia of Virology. 2008:29–37. doi:10.1016/B978-012374410-4.00585-9. (PMCID: PMC7150332).
105. James K. Immunoserology of infectious diseases. Clin Microbiol Rev. 1990;3(2):132–152. doi:10.1128/cmr.3.2.132. (PMCID: PMC358148).
106. Rao MKK, Kapila K, Gupta RM. Enzyme linked immunosorbent assays revisited. Med J Armed Forces India. 2017;53(1):45–51. doi:10.1016/S0377-1237(17)30644-5. (PMCID: PMC5530809).

 doi:10.20944/preprints202603.1630.v1

107. Aydin S, Emre E, Ugur K, et al. An overview of ELISA: a review and update on best laboratory practices for quantifying peptides and proteins in biological fluids. J Int Med Res. 2025;53(2):03000605251315913. doi:10.1177/03000605251315913. (PMCID: PMC11808753).
108. Boehringer HR, O'Farrell BJ. Lateral Flow Assays in Infectious Disease Diagnosis. Clin Chem. 2022;68(1):52–58. doi:10.1093/clinchem/hvab194.
109. Ren Q, Wang Y, Ma H, et al. Recent Advances in Lateral Flow Immunoassay for Rapid Diagnosis of Viral Diseases. Transbound Emerg Dis. 2026;2026:5701806. doi:10.1155/tbed/5701806.
110. Dinnes J, et al. Rapid, point-of-care antigen tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2025;2025(11):CD013705. doi:10.1002/14651858.CD013705.pub4.
111. Afzal N, Tariq N, Raza S, et al. Diagnostic Accuracy of Electro-Chemiluminescence Immunoassay Anti-SARS-CoV-2 Serological Test. Cureus. 2021;13(1):e12588. doi:10.7759/cureus.12588.
112. Liu K-T, Han Y-J, Wu G-H, et al. Overview of Neutralization Assays and International Standard for Detecting SARS-CoV-2 Neutralizing Antibody. Viruses. 2022;14(7):1560. doi:10.3390/v14071560.
113. Ward S, Lawford HLS, Sartorius B, Lau CL. Integrated Serosurveillance of Infectious Diseases Using Multiplex Bead Assays: A Systematic Review. Trop Med Infect Dis. 2025;10(1):19. doi:10.3390/tropicalmed10010019.
114. Carcelen AC, Monjane C, Bérubé S, et al. Multiplex bead assays enable integrated serological surveillance and reveal cross-pathogen vulnerabilities in Zambezia Province, Mozambique. Nat Commun. 2025;16:7946. doi:10.1038/s41467-025-62305-9.
115. Olivo PD, Ghatak S, et al. A Multiplex Bead Serology Panel For Vaccine-Preventable Diseases Using Dried Blood Spots. Risk Manag Healthc Policy. 2025;18:923–932. doi:10.2147/RMHP.S506025.
116. Lewinsohn DM, Leonard MK, LoBue PA, et al. Official ATS/IDSA/CDC Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clin Infect Dis. 2017;64(2):e1–e33. doi:10.1093/cid/ciw778.
117. Centers for Disease Control and Prevention. Clinical Testing Guidance for Tuberculosis: Interferon Gamma Release Assay (IGRA). Updated May 9, 2024. Accessed Feb 27, 2026. https://www.cdc.gov/tb/hcp/testing-diagnosis/interferon-gamma-release-assay.html
118. Deirmengian C, McLaren A, Higuera C, Levine BR. Physician Use of Multiple Criteria to Diagnose Periprosthetic Joint Infection May Be Less Accurate Than the Use of an Individual Test. Cureus. 2022;14(11):e31418. doi:10.7759/cureus.31418.
119. Land KJ, Boeras DI, Chen X-S, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol. 2019;4(1):46–54. doi:10.1038/s41564-018-0295-3.
120. Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect. 2010;16(8):1062–1069. doi:10.1111/j.1469-0691.2010.03279.x.
121. Kardjadj M. Advances in Point-of-Care Infectious Disease Diagnostics: Integration of Technologies, Validation, Artificial Intelligence, and Regulatory Oversight. Diagnostics (Basel). 2025;15(22):2845. doi:10.3390/diagnostics15222845.
122. Batac ALR, Marks M, Tucker JD, Peeling RW. Syphilis self-testing and implications for syphilis control and prevention. J Clin Microbiol. 2025 Dec 17;63(12):e0098225. doi:10.1128/jcm.00982-25. Epub 2025 Oct 21.
123. Anthony-George et al. Clinical Reviews in Microbiology. 2026. In press.
124. Otoo JA, Schlappi TS. REASSURED Multiplex Diagnostics: A Critical Review and Forecast. Biosensors (Basel). 2022;12(2):124. doi:10.3390/bios12020124.
125. Lehnert T, Gijs MAM. Microfluidic systems for infectious disease diagnostics. Lab Chip. 2024;24:1441–1493. doi:10.1039/D4LC00117F.
126. Vafadar A, et al. Advancements in microfluidic platforms for rapid biomarker diagnostics of infectious diseases. Microchem J. 2025;200:112296. doi:10.1016/j.microc.2024.112296.
127. Li W, et al. Review of paper-based microfluidic analytical devices for in-field testing of pathogens. Anal Chim Acta. 2023;1278:341614. doi:10.1016/j.aca.2023.341614.
128. García-Azuma R, et al. Unveiling the state of the art: a systematic review and meta-analysis of paper-based microfluidic devices. Front Bioeng Biotechnol. 2024;12:1421831. doi:10.3389/fbioe.2024.1421831.

 doi:10.20944/preprints202603.1630.v1

129. Reboud J, et al. Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. Proc Natl Acad Sci U S A. 2019;116(11):4834–4842. doi:10.1073/pnas.1812296116.
130. Liu H, et al. Paper-Based Microfluidic Chips for At-Home Point-of-Care Nucleic Acid Testing: Applications and Challenges. Diagnostics (Basel). 2026;16(2):251. doi:10.3390/diagnostics16020251.
131. Pawar AA, et al. Smartphone-based diagnostics for biosensing infectious human pathogens. Prog Biophys Mol Biol. 2023;180–181:120–130. doi:10.1016/j.pbiomolbio.2023.05.002.
132. Laksanasopin T, et al. A smartphone dongle for diagnosis of infectious diseases at the point of care. Sci Transl Med. 2015;7(273):273re1. doi:10.1126/scitranslmed.aaa0056.
133. Yang K, Peretz-Soroka H, Liu Y, Lin F. Novel developments in mobile sensing based on the integration of microfluidic devices and smartphones. Lab Chip. 2016;16:943–958. doi:10.1039/C5LC01524C.
134. Papadopoulos M, et al. Smartphone-Powered Automated Image Recognition Tool for Multianalyte Rapid Tests: Application to Infectious Diseases. Anal Chem. 2025;97(25):13340–13349. doi:10.1021/acs.analchem.5c01487.
135. Nxele SR, et al. Mobile-linked point-of-care diagnostics in community-based healthcare: a scoping review of user experiences. BMC Biomed Eng. 2024. doi:10.1186/s13690-024-01376-4.
136. Nakhjavani SA, Mirzajani H, Carrara S, Onbaşlı MC. Advances in biosensor technologies for infectious diseases detection. TrAC Trends Anal Chem. 2024;180:117979. doi:10.1016/j.trac.2024.117979.
137. Pashchenko O, Shelby T, Banerjee T, Santra S. A comparison of optical, electrochemical, magnetic, and colorimetric point-of-care biosensors for infectious disease diagnosis. ACS Infect Dis. 2018;4(8):1162–1178. doi:10.1021/acsinfecdis.8b00023.
138. Wachholz Junior D, Deroco PB, Hryniewicz BM, Kubota LT. Strategies for electrochemical point-of-care biosensors. Annu Rev Anal Chem. 2025;18:307–333. doi:10.1146/annurev-anchem-071124-103739.
139. Guo L, Zhao Y, Huang Q, et al. Electrochemical protein biosensors for disease marker detection: progress and opportunities. Microsyst Nanoeng. 2024;10:65. doi:10.1038/s41378-024-00700-w.
140. Parihar M, Niharika WN, Sahana, Biswas R, Dehury B, Mazumder N. Point-of-care biosensors for infectious disease diagnosis: recent updates and prospects. RSC Adv. 2025;15(36):29267–29283. doi:10.1039/D5RA03897A.
141. Ondevilla NAP, Liu P-W, Huang W-T, et al. A point-of-care electrochemical biosensor for the rapid and sensitive detection of biomarkers in murine models with LPS-induced sepsis. Biosens Bioelectron. 2024;254:116202. doi:10.1016/j.bios.2024.116202.
142. Špringer T, Bocková M, Slabý J, et al. Surface plasmon resonance biosensors and their medical applications. Biosens Bioelectron. 2025;278:117308. doi:10.1016/j.bios.2025.117308.
143. Prabowo BA, Purwidyantri A, Liu K-C. Surface plasmon resonance optical sensor: a review on light source technology. Biosensors (Basel). 2018;8(3):80. doi:10.3390/bios8030080.
144. Giarola JF, Estévez M-C, Lechuga LM. Plasmonic biosensors: towards fully operative detection platforms for biomedical application and its potential for the diagnosis of autoimmune diseases. TrAC Trends Anal Chem. 2024;176:117763. doi:10.1016/j.trac.2024.117763.
145. Hasan J, Bok S. Plasmonic fluorescence sensors in diagnosis of infectious diseases. Biosensors (Basel). 2024;14(3):130. doi:10.3390/bios14030130.
146. Truong H, Hoang T, Chung K, et al. Plasmonic biosensors and actuators for integrated point-of-care diagnostics. npj Biosensing. 2025;2:45. doi:10.1038/s44328-025-00050-1.
147. Wang J, Xu Z, Kotsifaki D. Plasmonic and metamaterial biosensors: a game-changer for virus detection. Sensors Diagn. 2023. doi:10.1039/D2SD00217E.
148. Ramirez-Priego P, Giarola JF, Andrés C, Antón A, Estévez M-C, Lechuga LM. Disposable point-of-care multiplexed plasmonic biosensor for rapid and specific identification of respiratory viruses. npj Biosensing. 2026;3:4. doi:10.1038/s44328-025-00071-w.
149. Ibrahim AU, et al. Nano-modified biosensors for detection of pathogenic diseases: the prospect of smart, multiplex and point-of-care testing. ADMET DMPK. 2025;13(4):2799. doi:10.5599/admet.2799.
150. Khan MQ, et al. Nanomaterial-based sensors for microbe detection: a review. Discover Nano. 2024;19:120. doi:10.1186/s11671-024-04065-x.

 doi:10.20944/preprints202603.1630.v1

151. Shahrashoob M, Dehshiri M, Yousefi V, et al. Optical and electrochemical biosensors for detection of pathogens using metal nanoclusters: a systematic review. Biosensors (Basel). 2025;15(7):460. doi:10.3390/bios15070460.
152. Ardila CM. Advancing healthcare through laboratory on a chip technology: transforming microorganism identification and diagnostics. World J Clin Cases. 2025;13(3):97737. doi:10.12998/wjcc.v13.i3.97737.
153. Mejías A, Ramilo O. Host transcriptomics for diagnosis of infectious diseases: from bedside to bench and back. Front Pediatr. 2021;9:685300.
154. Chawla DG, et al. Benchmarking transcriptional host response signatures for infection diagnosis. Cell Syst. 2022;13(7):521–540.e5. doi:10.1016/j.cels.2022.05.008.
155. Rao AM, et al. A robust host-response-based signature for bacterial versus viral infection and its limitations for intracellular bacteria. Cell Rep Med. 2022;3(12):100812. doi:10.1016/j.xcrm.2022.100812.
156. Sweeney TE, Azad TD, Donato M, et al. Unsupervised analysis of transcriptomics in sepsis identifies diagnostic and prognostic signatures. Nat Commun. 2018;9:1241. doi:10.1038/s41467-018-03078-2.
157. Moore AR, et al. A consensus immune dysregulation framework for sepsis endotypes and clinical translation. Nat Med. 2025. doi:10.1038/s41591-025-03956-5.
158. Birhanu AG, et al. Mass spectrometry-based proteomics as an emerging tool in clinical microbiology. Front Microbiol. 2023;14:1214699. doi:10.3389/fmicb.2023.1214699.
159. Lee H, et al. LC–MS-based proteomic analyses of body fluids to diagnose infectious diseases. Int J Mol Sci. 2022;23(4):2187. doi:10.3390/ijms23042187.
160. Kartowikromo KY, et al. Advancements in ion mobility–based diagnostics for infectious diseases. Front Microbiol. 2025.
161. Grossegesse M, et al. vPro-MS enables identification of human-pathogenic viruses from patient samples by untargeted proteomics. Nat Commun. 2025. doi:10.1038/s41467-025-62469-4.
162. Sadaka K, et al. Metabolomics of volatile organic compounds in infectious diseases: advances and applications. TrAC Trends Anal Chem. 2024;183:116507. doi:10.1016/j.trac.2024.116507.
163. Hogan CA, et al. Comprehensive metabolomics and machine learning for direct identification of SARS-CoV-2 and other viruses from upper respiratory samples. Clin Chem. 2025. (PMID: 41065362).
164. Rahman M, et al. Metabolomics in infectious diseases and vaccine response. Front Mol Biosci. 2026.
165. Mohr AE, et al. Navigating challenges and opportunities in multi-omics integration for biomedical applications. Biomedicines. 2024;12(7):1496. doi:10.3390/biomedicines12071496.
166. Babu M, et al. Multi-omics profiling for health: opportunities and challenges for integrated analysis. Mol Cell Proteomics. 2023;22(8):100579. doi:10.1016/j.mcpro.2023.100579.
167. Ma J, et al. Role of multi-omics in the diagnosis of COVID-19 and precision medicine: a review. OMICS. 2022;26(9):475–492. doi:10.1089/omi.2022.0128.
168. Pitarch A, et al. Omics and multiomics-based diagnostics for invasive candidiasis. Front Cell Infect Microbiol. 2025.
169. Patterson TF, Thompson GR III, Denning DW, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):e1–e60. doi:10.1093/cid/ciw326.
170. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. β-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clin Infect Dis. 2011;52(6):750–770. doi:10.1093/cid/ciq206.
171. Guo YL, Chen YQ, Wang K, Qin SM, Wu C, Kong JL. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate meta-analysis and systematic review. Chest. 2010;138(4):817–824. doi:10.1378/chest.10-0488.
172. Sinclair A, Xie X, Teltscher M, Dendukuri N. Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by Streptococcus pneumoniae. J Clin Microbiol. 2013;51(7):2303–2310. doi:10.1128/JCM.00137-13.
173. World Health Organization. Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV: policy update. Geneva: WHO; 2019.

 doi:10.20944/preprints202603.1630.v1

174. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis. 2004;39(2):206–217. doi:10.1086/421997.
175. Kamat IS, Ramachandran V, Eswaran H, Guffey D, Musher DM. Procalcitonin to distinguish viral from bacterial pneumonia: a systematic review and meta-analysis. Clin Infect Dis. 2020;70(3):538–542. doi:10.1093/cid/ciz545.
176. Norman-Bruce H, Umana E, Mills C, et al. Diagnostic test accuracy of procalcitonin and C-reactive protein for predicting invasive and serious bacterial infections in young febrile infants: systematic review and meta-analysis. Lancet Child Adolesc Health. 2024;8(5):358–368. doi:10.1016/S2352-4642(24)00021-X.
177. Miller RR III, Lopansri BK, Burke JP, et al. Validation of a host response assay, SeptiCyte LAB, for discriminating sepsis from systemic inflammatory response syndrome in the ICU. Am J Respir Crit Care Med. 2018;198(7):903–913. doi:10.1164/rccm.201712-2472OC.
178. Balk R, Esper AM, Martin GS, et al. Validation of SeptiCyte RAPID to Discriminate Sepsis from Non-Infectious Systemic Inflammation. J Clin Med. 2024;13(5):1194. doi:10.3390/jcm13051194.
179. Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia: An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45–e67. doi:10.1164/rccm.201908-1581ST.
180. Lan HM, Wu CC, Liu SH, et al. Comparison of the diagnostic accuracies of various biomarkers and scoring systems for sepsis: a systematic review and Bayesian diagnostic test accuracy network meta-analysis. J Crit Care. 2025;88:155087. doi:10.1016/j.jcrc.2025.155087.
181. Sanders S, Barnett A, Correa-Velez I, Coulthard M, Doust J. Systematic review of the diagnostic accuracy of C-reactive protein to detect bacterial infection in nonhospitalized infants and children with fever. J Pediatr. 2008;153(4):570–574. doi:10.1016/j.jpeds.2008.04.023.
182. Jerome E, McPhail MJ, Menon K. Diagnostic accuracy of procalcitonin and interleukin-6 for postoperative infection in major gastrointestinal surgery: a systematic review and meta-analysis. Ann R Coll Surg Engl. 2022;104(8):561–570. doi:10.1308/rcsann.2022.0053.
183. Dhana A, Hamada Y, Kengne APK, et al. Diagnostic accuracy of WHO screening criteria to guide lateral-flow lipoarabinomannan testing among HIV-positive inpatients: a systematic review and individual participant data meta-analysis. J Infect. 2022;85(1):40–48. doi:10.1016/j.jinf.2022.05.010.
184. World Health Organization. Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Therapy. Geneva: WHO; 2017.
185. Holmberg SD, Lu M, Rupp LB, et al. Noninvasive serum fibrosis markers for screening and staging chronic hepatitis C virus patients in a large US cohort. Clin Infect Dis. 2013;57(2):240–246. doi:10.1093/cid/cit245.
186. Lawn SD, Gupta-Wright A. Detection of lipoarabinomannan (LAM) in urine is indicative of disseminated TB with renal involvement in patients living with HIV and advanced immunodeficiency: evidence and implications. Trans R Soc Trop Med Hyg. 2016;110(3):180–185. doi:10.1093/trstmh/trw008.
187. World Health Organization. Guidelines for diagnosing and managing disseminated histoplasmosis among people living with HIV. Geneva: WHO; 2020.
188. Tang YW, Li H, Durkin MM, et al. Urine polymerase chain reaction is not as sensitive as urine antigen for the diagnosis of disseminated histoplasmosis. Diagn Microbiol Infect Dis. 2006;54(4):283–287. doi:10.1016/j.diagmicrobio.2005.10.008.
189. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67(4):1560–1599. doi:10.1002/hep.29800.
190. Conners EE, Panagiotakopoulos L, Hofmeister MG, et al. Screening and Testing for Hepatitis B Virus Infection: CDC Recommendations—United States, 2023. MMWR Recomm Rep. 2023;72(1):1–25. doi:10.15585/mmwr.rr7201a1.
191. Wake RM, Britz E, Sriruttan C, et al. High cryptococcal antigen titers in blood are predictive of subclinical cryptococcal meningitis among HIV-infected patients. Clin Infect Dis. 2018;66(5):686–692. doi:10.1093/cid/cix872.

 doi:10.20944/preprints202603.1630.v1

192. Nacher M, Adenis A, Blanchet D, et al. Histoplasma capsulatum antigen detection tests as an essential diagnostic tool for patients with advanced HIV disease in low and middle income countries: a systematic review of diagnostic accuracy studies. PLoS Negl Trop Dis. 2018;12(10):e0006802. doi:10.1371/journal.pntd.0006802.
193. Centers for Disease Control and Prevention. Clinical Testing and Diagnosis for Hepatitis B. CDC website; updated 2025 (accessed Feb 27, 2026).
194. Hage CA, Kirsch EJ, Stump TE, et al. Histoplasma antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. Clin Vaccine Immunol. 2011;18(4):661–666. doi:10.1128/CVI.00389-10.
195. Mercier T, Guldentops E, Lagrou K, Maertens J. Galactomannan, a surrogate marker for outcome in invasive aspergillosis: finally coming of age. Front Microbiol. 2018;9:661. doi:10.3389/fmicb.2018.00661.
196. Chai LYA, Kullberg BJ, Johnson EM, et al. Early serum galactomannan trend as a predictor of outcome of invasive aspergillosis. J Clin Microbiol. 2012;50(7):2330–2336. doi:10.1128/JCM.06513-11.
197. Wallis RS, Wang C, Meyer D, Thomas N. Month 2 culture status and treatment duration as predictors of tuberculosis relapse risk in a meta-regression model. PLoS One. 2013;8(8):e71116. doi:10.1371/journal.pone.0071116.
198. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021;47(11):1181–1247. doi:10.1007/s00134-021-06506-y.
199. Schuetz P, Wirz Y, Sager R, et al. Effect of procalcitonin-guided antibiotic treatment on mortality in acute respiratory infections: a patient level meta-analysis. Lancet Infect Dis. 2018;18(1):95–107. doi:10.1016/S1473-3099(17)30592-3.
200. Bouadma L, Luyt C-E, Tubach F, et al. Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicentre randomised controlled trial. Lancet. 2010;375(9713):463–474. doi:10.1016/S0140-6736(09)61879-1.
201. Dark P, Hossain A, McAuley DF, et al. Biomarker-Guided Antibiotic Duration for Hospitalized Patients With Suspected Sepsis: The ADAPT-Sepsis Randomized Clinical Trial. JAMA. 2025;333(8):682–693. doi:10.1001/jama.2024.26458.
202. Wirz Y, Meier MA, Bouadma L, et al. Effect of procalcitonin-guided antibiotic treatment on clinical outcomes in intensive care unit patients with infection and sepsis: a patient-level meta-analysis of randomized trials. Crit Care. 2018;22:191. doi:10.1186/s13054-018-2125-7.
203. Huang DT, Yealy DM, Filbin MR, et al. Procalcitonin-Guided Use of Antibiotics for Lower Respiratory Tract Infection. N Engl J Med. 2018;379(3):236–249. doi:10.1056/NEJMoa1802670.
204. von Dach E, Albrich WC, Brunel AS, et al. Effect of C-Reactive Protein–Guided Antibiotic Treatment Duration, 7-Day Treatment, or 14-Day Treatment on 30-Day Clinical Failure Rate in Patients With Uncomplicated Gram-Negative Bacteremia: A Randomized Clinical Trial. JAMA. 2020;323(21):2160–2169. doi:10.1001/jama.2020.6348.
205. Dias RF, de Paula ACRB, Hasparyk UG, et al. Use of C-reactive protein to guide the antibiotic therapy in hospitalized patients: a systematic review and meta-analysis. BMC Infect Dis. 2023;23(1):276. doi:10.1186/s12879-023-08255-3.
206. U.S. Food and Drug Administration. Microbiological Data for Systemic Antibacterial Drug Products—Development, Analysis, and Presentation. Guidance for Industry. Silver Spring, MD: FDA; 2018.
207. Weiss E, Zahar J-R, Alder J, et al. Elaboration of consensus clinical endpoints to evaluate antimicrobial treatment efficacy in future hospital-acquired/ventilator-associated bacterial pneumonia clinical trials. Clin Infect Dis. 2019;69(11):1912–1918. doi:10.1093/cid/ciz093.
208. Theron G, Venter R, Smith L, et al. False-positive Xpert MTB/RIF results in retested patients with previous tuberculosis: frequency, profile, and prospective clinical outcomes. J Clin Microbiol. 2018;56:e01696-17. doi:10.1128/JCM.01696-17.
209. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis. 2018;18(1):76–84. doi:10.1016/S1473-3099(17)30691-6.

 doi:10.20944/preprints202603.1630.v1

210. Dalrymple U, Arambepola R, Gething PW, Cameron E. How long do rapid diagnostic tests remain positive after anti-malarial treatment? Malar J. 2018;17:228. doi:10.1186/s12936-018-2371-9.
211. Lazenby GB, Korte JE, Tillman S, et al. A recommendation for timing of repeat Chlamydia trachomatis test following infection and treatment in pregnant and nonpregnant women. Int J STD AIDS. 2017;28(9):902–909. doi:10.1177/0956462416680438.
212. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe. 2021;2(1):e13–e22. doi:10.1016/S2666-5247(20)30172-5.
213. Henderson DK, Weber DJ, Babcock H, et al. The perplexing problem of persistently PCR-positive personnel. Infect Control Hosp Epidemiol. 2021;42(2):203–204. doi:10.1017/ice.2020.343.
214. Espinosa-Pereiro J, Alagna R, Saluzzo F, et al. A systematic review of potential biomarkers for bacterial burden and treatment efficacy assessment in tuberculosis platform-based clinical trials. J Infect Dis. 2024;229(5):1584–1595. doi:10.1093/infdis/jiad482.
215. Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenza. Clin Infect Dis. 2019;68(6):e1–e47. doi:10.1093/cid/ciy866.
216. Centers for Disease Control and Prevention. Overview of Influenza Testing Methods. Updated Dec 19, 2025. Accessed Feb 27, 2026.
217. Centers for Disease Control and Prevention. Diagnostic Testing for RSV. Updated Feb 24, 2026. Accessed Feb 27, 2026.
218. Hanson KE, Caliendo AM, Arias CA, et al. The Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19: Molecular Diagnostic Testing (January 2021). Clin Infect Dis. 2024;78(7):e170–e207. doi:10.1093/cid/ciab048.
219. Centers for Disease Control and Prevention; Association of Public Health Laboratories. 2018 Quick Reference Guide: Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens. 2018.
220. Branson BM, Owen SM, Wesolowski LG, et al. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. CDC; 2014. doi:10.15620/cdc.23447.
221. Panel on Antiretroviral Guidelines for Adults and Adolescents. Laboratory Testing for Initial Assessment and Monitoring of People With HIV Receiving ART. Updated Sep 25, 2025. Accessed Feb 27, 2026.
222. World Health Organization. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection. WHO; 2024.
223. Centers for Disease Control and Prevention. Clinical Screening and Diagnosis for Hepatitis C. Updated Jan 31, 2025. Accessed Feb 27, 2026.
224. Cartwright EJ, Patel P, Kamili S, Wester C. Updated Operational Guidance for Implementing CDC's Recommendations on Testing for Hepatitis C Virus Infection. MMWR Morb Mortal Wkly Rep. 2023;72(28):766–768. doi:10.15585/mmwr.mm7228a2.
225. Centers for Disease Control and Prevention. Clinical Testing Guidance for Dengue. Updated Aug 26, 2025. Accessed Feb 27, 2026.
226. Sharp TM, Fischer M, Muñoz-Jordán JL, et al. Dengue and Zika Virus Diagnostic Testing for Patients with a Clinically Compatible Illness and Risk for Infection with Both Viruses. MMWR Recomm Rep. 2019;68(RR-1):1–10. doi:10.15585/mmwr.rr6801a1.
227. Pillay K, Keddie SH, Fitchett E, et al. Evaluating the performance of common reference laboratory tests for acute dengue diagnosis: a systematic review and meta-analysis of RT-PCR, NS1 ELISA, and IgM ELISA. Lancet Microbe. 2025;6(7):101088. doi:10.1016/j.lanmic.2025.101088.
228. Centers for Disease Control and Prevention. Clinical Testing and Diagnosis for Zika Virus Disease. Updated Nov 19, 2025. Accessed Feb 27, 2026.
229. Centers for Disease Control and Prevention. Clinical Testing and Diagnosis for Chikungunya Virus Disease. Updated Nov 19, 2025. Accessed Feb 27, 2026.
230. Johnson BW, Russell BJ, Goodman CH. Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays. J Infect Dis. 2016;214(Suppl 5):S471–S474. doi:10.1093/infdis/jiw274.

 doi:10.20944/preprints202603.1630.v1

231. Centers for Disease Control and Prevention. Clinical Testing and Diagnosis for West Nile Virus Disease. Updated Nov 19, 2025. Accessed Feb 27, 2026.
232. Chan KR, Ismail AA, Thergarajan G, et al. Serological cross-reactivity among common flaviviruses. Front Cell Infect Microbiol. 2022;12:975398. doi:10.3389/fcimb.2022.975398.
233. Centers for Disease Control and Prevention. Updated Interim Guidance for Health Departments on Testing and Reporting for Oropouche Virus Disease. Updated Nov 19, 2025. Accessed Feb 27, 2026.
234. Pan American Health Organization. Recommendations for the Detection and Surveillance of Oropouche. 2024. Accessed Feb 27, 2026.
235. Lamy B, Dargière S, Arendrup MC, Parienti JJ, Tattevin P. How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state-of-the-art. Front Microbiol. 2016;7:697. doi:10.3389/fmicb.2016.00697.
236. Clinical and Laboratory Standards Institute (CLSI). Principles and Procedures for Blood Cultures. CLSI guideline M47, 2nd ed. Wayne, PA: CLSI; 2022.
237. Fabre V, Carroll KC, Cosgrove SE. Blood culture utilization in the hospital setting: a call for diagnostic stewardship. J Clin Microbiol. 2022;60(3):e01005-21. doi:10.1128/JCM.01005-21.
238. Wang Y, Lindsley K, Bleak TC, et al. Performance of molecular tests for diagnosis of bloodstream infections in the clinical setting: a systematic literature review and meta-analysis. Clin Microbiol Infect. 2025;31(3):360–372. doi:10.1016/j.cmi.2024.12.007.
239. Peri AM, Ling W, Furuya-Kanamori L, Harris PNA, Paterson DL. Performance of BioFire Blood Culture Identification 2 Panel (BCID2) for detection of bloodstream pathogens and resistance markers: systematic review and meta-analysis of diagnostic test accuracy studies. BMC Infect Dis. 2022;22(1):794. doi:10.1186/s12879-022-07772-x.
240. Nguyen MH, Clancy CJ, Pasculle AW, et al. Performance of the T2Bacteria Panel for diagnosing bloodstream infections: a diagnostic accuracy study. Ann Intern Med. 2019;170(12):845–852. doi:10.7326/M18-2772.
241. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis – rapid diagnostics for tuberculosis detection. 3rd ed. WHO; 2024.
242. Schwab TC, Perrig L, et al. Targeted next-generation sequencing to diagnose drug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2024. doi:10.1016/S1473-3099(24)00263-9.
243. Papp JR, Park IU, Fakile Y, et al. CDC Laboratory Recommendations for Syphilis Testing, United States, 2024. MMWR Recomm Rep. 2024;73(RR-1).
244. Centers for Disease Control and Prevention. Gonorrhea Laboratory Information. Updated Mar 19, 2024. Accessed Feb 27, 2026.
245. Ellis O, Hemarajata P, Shahkolahi A, et al. A multisite implementation of a real-time PCR assay to predict ciprofloxacin susceptibility in Neisseria gonorrhoeae. Diagn Microbiol Infect Dis. 2019;94(3):213–217. doi:10.1016/j.diagmicrobio.2018.12.018.
246. Centers for Disease Control and Prevention. Drug-Resistant Gonorrhea. Updated Feb 15, 2024. Accessed Feb 27, 2026.
247. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the EORTC/MSGERC. Clin Infect Dis. 2020;71(6):1367–1376. doi:10.1093/cid/ciz1008.
248. Patterson TF, Thompson GR 3rd, Denning DW, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):e1–e60. doi:10.1093/cid/ciw326.
249. Pappas PG, Kauffman CA, Andes DR, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;62(4):e1–e50. doi:10.1093/cid/civ933.
250. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis. 2010;50(3):291–322. doi:10.1086/649858.
251. Cornely OA, Alastruey-Izquierdo A, Arenz D, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the

 doi:10.20944/preprints202603.1630.v1

Mycoses Study Group Education and Research Consortium. Lancet Infect Dis. 2019;19(12):e405–e421. doi:10.1016/S1473-3099(19)30312-3.

252. Arvanitis M, Anagnostou T, Mylonakis E. Galactomannan and Polymerase Chain Reaction-Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis. Clin Infect Dis. 2015;61(8):1263–1272. doi:10.1093/cid/civ555.
253. Heng SC, Morrissey O, Chen SCA, et al. Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematology patients: a systematic review and meta-analysis. Crit Rev Microbiol. 2015;41(1):124–134. doi:10.3109/1040841X.2013.804033.
254. He S, Hang J-P, Zhang L, Wang F, Zhang D-C, Gong F-H. A systematic review and meta-analysis of diagnostic accuracy of serum 1,3-β-D-glucan for invasive fungal infection: Focus on cutoff levels. J Microbiol Immunol Infect. 2015;48(4):351–361. doi:10.1016/j.jmii.2014.06.009.
255. Huang H-R, Fan L-C, Rajbanshi B, Xu J-F. Evaluation of a new cryptococcal antigen lateral flow immunoassay in serum, cerebrospinal fluid and urine for the diagnosis of cryptococcosis: a meta-analysis and systematic review. PLoS ONE. 2015;10(5):e0127117. doi:10.1371/journal.pone.0127117.
256. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis. 2009;9(2):89–96. doi:10.1016/S1473-3099(09)70019-2.
257. Clancy CJ, Pappas PG, Vazquez J, et al. Detecting Infections Rapidly and Easily for Candidemia Trial, Part 2 (DIRECT2): A Prospective, Multicenter Study of the T2Candida Panel. Clin Infect Dis. 2018;66(11):1678–1686. doi:10.1093/cid/cix1095.
258. Millon L, Caillot D, Berceanu A, et al. Evaluation of Serum Mucorales Polymerase Chain Reaction (PCR) for the Diagnosis of Mucormycoses: The MODIMUCOR Prospective Trial. Clin Infect Dis. 2022;75(5):777–785. doi:10.1093/cid/ciab1066.
259. World Health Organization. Diagnostic testing for malaria. WHO. Accessed Feb 27, 2026.
260. World Health Organization. Response plan to pfhrp2 gene deletions, second edition. WHO; 2024. Accessed Feb 27, 2026.
261. Gatton ML, Chaudhry A, Glenn J, et al. Impact of Plasmodium falciparum gene deletions on malaria rapid diagnostic test performance. Malar J. 2020;19:392. doi:10.1186/s12936-020-03460-w.
262. Boelaert M, Verdonck K, Menten J, et al. Rapid tests for the diagnosis of visceral leishmaniasis in patients with suspected disease. Cochrane Database Syst Rev. 2014;(6):CD009135. doi:10.1002/14651858.CD009135.pub2.
263. Bharadwaj M, Bengtson M, Golverdingen M, Waling L, Dekker C. Diagnosing point-of-care diagnostics for neglected tropical diseases. PLoS Negl Trop Dis. 2021;15(6):e0009405. doi:10.1371/journal.pntd.0009405.
264. World Health Organization. WHO guideline on control and elimination of human schistosomiasis. WHO; 2022. Accessed Feb 27, 2026.
265. Alemu G, Nibret E. Evaluation of the Urine POC-CCA Test Accuracy in the Detection of Schistosoma mansoni Infection: A Systematic Review and Meta-Analysis. J Trop Med. 2024;2024:5531687. doi:10.1155/2024/5531687.
266. Tamarozzi F, Ursini T, Hoekstra PT, et al. Evaluation of microscopy, serology, circulating anodic antigen (CAA), and eosinophil counts for the follow-up of migrants with chronic schistosomiasis: a prospective cohort study. Parasites & Vectors. 2021;14:149. doi:10.1186/s13071-021-04655-z.
267. Hagström L, Pereira Marques AL, Nitz N, Hecht MM. The use of qPCR in human Chagas disease: a systematic review. Expert Rev Mol Diagn. 2019;19(10):875–894. doi:10.1080/14737159.2019.1659729.
268. Pan American Health Organization / World Health Organization. Guidelines for the diagnosis and treatment of Chagas disease. PAHO/WHO; 2018. Accessed Feb 27, 2026.
269. Zrein M, Granjon E, Gueyffier L, et al. A novel antibody surrogate biomarker to monitor parasite persistence in Trypanosoma cruzi-infected patients. PLoS Negl Trop Dis. 2018;12(2):e0006226. doi:10.1371/journal.pntd.0006226.
270. Centers for Disease Control and Prevention. CDC DPDx: Serum/Plasma Specimens—Antibody Detection (limitations and persistence after cure). CDC. Accessed Feb 27, 2026.

 doi:10.20944/preprints202603.1630.v1

271. World Health Organization. Guidelines for the treatment of human African trypanosomiasis. WHO; 2024. Accessed Feb 27, 2026.
272. Vincent IM, Daly R, Courtioux B, et al. Metabolomics Identifies Multiple Candidate Biomarkers to Diagnose and Stage Human African Trypanosomiasis. PLoS Negl Trop Dis. 2016;10(12):e0005140. doi:10.1371/journal.pntd.0005140.
273. Clinical and Laboratory Standards Institute. EP17: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed. CLSI; 2012.
274. Clinical and Laboratory Standards Institute. EP05: Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed. CLSI; 2014.
275. Clinical and Laboratory Standards Institute. EP06: Evaluation of Linearity of Quantitative Measurement Procedures. 2nd ed. CLSI; 2020.
276. Clinical and Laboratory Standards Institute. EP07: Interference Testing in Clinical Chemistry. 3rd ed. CLSI; 2018.
277. Clinical and Laboratory Standards Institute. PRE04: Handling, Transport, Processing, and Storage of Blood Specimens for Routine Laboratory Examinations. CLSI; 2023.
278. U.S. Food and Drug Administration. Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA - Class II Special Controls Guidance for Industry and FDA Staff. FDA; 2009.
279. Hasan MR, Tan R, Al-Rawahi GN, Thomas E, Tilley P. Short-term stability of pathogen-specific nucleic acid targets in clinical samples. J Clin Microbiol. 2012;50(12):4147–4150.
280. Murugesan K, Hogan CA, Palmer Z, et al. Investigation of preanalytical variables impacting pathogen cell-free DNA in blood and urine. J Clin Microbiol. 2019;57(11):e00782-19.
281. Banoo S, Bell D, Bossuyt P, et al. Evaluation of diagnostic tests for infectious diseases: general principles. Nat Rev Microbiol. 2006;4(12 Suppl):S20–S32. doi:10.1038/nrmicro1570.
282. Altman DG, Bland JM. Diagnostic tests 2: predictive values. BMJ. 1994;309:102. doi:10.1136/bmj.309.6947.102.
283. Deeks JJ, Altman DG. Diagnostic tests 4: likelihood ratios. BMJ. 2004;329(7458):168–169. doi:10.1136/bmj.329.7458.168.
284. Hajian-Tilaki K. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. Caspian J Intern Med. 2013;4(2):627–635.
285. Patel R, Tsalik EL, Evans S, Fowler VG, Doernberg SB; Antibacterial Resistance Leadership Group. Clinically Adjudicated Reference Standards for Evaluation of Infectious Diseases Diagnostics. Clin Infect Dis. 2023;76(5):938–943. doi:10.1093/cid/ciac829.
286. Limmathurotsakul D, Turner EL, Wuthiekanun V, et al. Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. Clin Infect Dis. 2012;55(3):322–331. doi:10.1093/cid/cis403.
287. Lijmer JG, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, van der Meulen JH, Bossuyt PM. Empirical evidence of design-related bias in studies of diagnostic tests. JAMA. 1999;282(11):1061–1066. doi:10.1001/jama.282.11.1061.
288. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J. Designing studies to ensure that estimates of test accuracy are transferable. BMJ. 2002;324(7338):669–671. doi:10.1136/bmj.324.7338.669.
289. Bujang MA. An Elaboration on Sample Size Planning for Performing a One-Sample Sensitivity and Specificity Analysis by Basing on Calculations on a Specified 95% Confidence Interval Width. Diagnostics (Basel). 2023;13(8):1390. doi:10.3390/diagnostics13081390.
290. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.
291. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, the PRISMA-DTA Group, et al. Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018;319(4):388–396. doi:10.1001/jama.2017.19163.
292. Ness M, Kwakye-Nuako G, Godinho J, Edens JR, Laro JA, Agyapong J, et al. Small molecule biomarkers in infectious disease. Adv Clin Chem. 2026;131:141–176. doi:10.1016/bs.acc.2025.10.006.

 doi:10.20944/preprints202603.1630.v1

293. Gavina K, Franco LC, Khan H, Lavik J-P, Relich RF. Molecular point-of-care devices for the diagnosis of infectious diseases in resource-limited settings: A review of the current landscape, technical challenges, and clinical impact. J Clin Virol. 2023;169:105613. doi:10.1016/j.jcv.2023.105613.
294. Fleming KA, Horton S, Wilson ML, Atun R, DeStigter K, Flanigan J, et al. The Lancet Commission on diagnostics: transforming access to diagnostics. Lancet. 2021;398(10315):1997–2050. doi:10.1016/S0140-6736(21)00673-5.
295. Therapeutic Goods Administration. Meeting clinical evidence requirements for in-vitro diagnostic (IVD) medical devices. TGA; 2020. Updated 2024.
296. World Health Organization. Post-market surveillance of in vitro diagnostics. WHO; 2015.
297. World Health Organization. Selection of essential in vitro diagnostics at country level: using the WHO Model List of Essential In Vitro Diagnostics to develop and update a national list of essential in vitro diagnostics. WHO; 2021.
298. Hanson KE, Tsalik EL. Host Immune Response Profiling for the Diagnosis of Infectious Diseases. J Infect Dis. 2025;232(5):1029–1039. doi:10.1093/infdis/jiaf428.
299. Miglietta L, Rawson TM, Galiwango R, et al. Artificial intelligence and infectious disease diagnostics: state of the art and future perspectives. Lancet Infect Dis. 2025. doi:10.1016/S1473-3099(25)00354-8.
300. Talianu A, Fraser-Krauss O, Bolton W, et al. Artificial intelligence for improving decision-making in bacterial infection management: a narrative review. J Antimicrob Chemother. 2026;81(1):dkaf470. doi:10.1093/jac/dkaf470.
301. Tran NK, Albahra S, Rashidi H, May L. Innovations in infectious disease testing: Leveraging COVID-19 pandemic technologies for the future. Clin Biochem. 2023;117:10–15. doi:10.1016/j.clinbiochem.2021.12.011.
302. Gonzalez Cruz C, Sodawalla HM, Mohanakumar T, Bansal S. Extracellular Vesicles as Biomarkers in Infectious Diseases. Biology (Basel). 2025;14(2):182. doi:10.3390/biology14020182.
303. Mutavhatsindi H, Manyelo CM, Snyders CI, et al. Baseline and end-of-treatment host serum biomarkers predict relapse in adults with pulmonary tuberculosis. J Infect. 2024;89(1):106173. doi:10.1016/j.jinf.2024.106173.
304. Wallis RS, Doherty TM, Onyebujoh P, et al. Biomarkers for tuberculosis disease activity, cure, and relapse. Lancet Infect Dis. 2009;9(3):162–172. doi:10.1016/S1473-3099(09)70042-8.
305. Grossberg AN, Koza LA, Ledreux A, et al. A multiplex chemiluminescent immunoassay for serological profiling of COVID-19-positive symptomatic and asymptomatic patients. Nat Commun. 2021;12:740. doi:10.1038/s41467-021-21040-7.
306. Gong MM, Sinton D. Turning the Page: Advancing Paper-Based Microfluidics for Broad Diagnostic Application. Chem Rev. 2017;117(12):8447–8480. doi:10.1021/acs.chemrev.7b00024.
307. Ramilo O, Mejías A. Shifting the paradigm: host gene signatures for diagnosis of infectious diseases. Clin Infect Dis. 2009;49(3):448–450. doi:10.1086/600301.
308. Liu J, et al. Artificial intelligence with mass spectrometry-based omics strategies in infectious diseases: current state and future trends. Crit Rev Microbiol. 2024. doi:10.1080/1040841X.2024.2340132.
309. Drain PK, Niu X, Shapiro AE, et al. Real-world diagnostic accuracy of lipoarabinomannan in three non-sputum biospecimens for pulmonary tuberculosis disease. eBioMedicine. 2024;108:105353. doi:10.1016/j.ebiom.2024.105353.
310. Åkerlund A, Jonasson E, Matuschek E, et al. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories. J Antimicrob Chemother. 2020;75(11):3230–3238. doi:10.1093/jac/dkaa333.
311. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0. 2024.
312. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100, 36th ed. Wayne, PA: CLSI; 2026.
313. World Health Organization. Global antibiotic resistance surveillance report 2025. WHO; 2025.
314. World Health Organization. Global Antimicrobial Resistance and Use Surveillance System (GLASS). Accessed Feb 27, 2026.

 doi:10.20944/preprints202603.1630.v1

315. Tamma PD, Heil EL, Justo JA, et al. Infectious Diseases Society of America 2024 Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections. Clin Infect Dis. 2024. doi:10.1093/cid/ciae403.
316. Banerjee R, Humphries RM. Rapid antimicrobial susceptibility testing methods for blood cultures and their clinical impact. Front Med (Lausanne). 2021;8:635831. doi:10.3389/fmed.2021.635831.
317. van Belkum A, Burnham C-AD, Rossen JWA, Mallard F, Rochas O, Dunne WM Jr. Innovative and rapid antimicrobial susceptibility testing systems. Nat Rev Microbiol. 2020;18:299–311. doi:10.1038/s41579-020-0327-x.
318. Hu Y, Zhao Y, Zhang Y, Chen W, Zhang H, Jin X. Cell-free DNA: a promising biomarker in infectious diseases. Trends Microbiol. 2025;33(4):421–433. doi:10.1016/j.tim.2024.06.005.
319. Taylor, T.A.; Tobin, C.M.; Unakal, C.G. Staphylococcus aureus Infection. In StatPearls; StatPearls Publishing: Treasure Island, FL, USA.
320. Jenks, J.D.; Tobin, E.H. Candidiasis. In StatPearls; StatPearls Publishing: Treasure Island, FL, USA; 2026.
321. Wisconsin Department of Health Services. Streptococcus pyogenes (Group A Streptococcus). Available online: https://www.dhs.wisconsin.gov/disease/streptococcus.htm
322. Loesche, W.J. Role of Streptococcus mutans in human dental decay. EBioMedicine 2017, 24, 205–215.
323. Brook, I. Actinomycosis: Diagnosis and management. Aust. N. Z. J. Obstet. Gynaecol. 1989, 29, 445–449.
324. Brennan, C.A.; Garrett, W.S. Fusobacterium nucleatum—symbiont, opportunist and oncobacterium. Nat. Rev. Microbiol. 2019, 17, 156–166.
325. MacNeil, J.R.; et al. Current epidemiology and trends in invasive Haemophilus influenzae disease. Open Forum Infect. Dis. 2014, 1, ofu044.
326. Centers for Disease Control and Prevention (CDC). Meningococcal Disease. Available online: https://www.cdc.gov/meningococcal
327. Centers for Disease Control and Prevention (CDC). Pneumococcal Disease. Available online: https://www.cdc.gov/pneumococcal
328. New York State Department of Health. Legionellosis (Legionnaires' Disease) Fact Sheet. Available online: https://www.health.ny.gov/diseases/communicable/legionellosis
329. Ashurst, J.V.; Dawson, A. Klebsiella pneumoniae. In StatPearls; StatPearls Publishing: Treasure Island, FL, USA.
330. Lessa, F.C.; Mu, Y.; Bamberg, W.M.; et al. Burden of Clostridioides difficile infection in the United States. J. Clin. Microbiol. 2014, 52, 2406–2409.
331. Centers for Disease Control and Prevention (CDC). Rabies. Available online: https://www.cdc.gov/rabies
332. World Health Organization (WHO). Group B Streptococcus Infection. Available online: https://www.who.int
333. Cleveland Clinic. Gardnerella vaginalis. Available online: https://my.clevelandclinic.org
334. Chan, P.A.; Robinette, A.; Montgomery, M.; et al. Extragenital infections caused by Chlamydia trachomatis. Int. J. STD AIDS 2021, 32, 510–516.