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REVIEW

Emerging Technologies for Molecular Diagnosis of Sepsis

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SUMMARY Rapid and accurate profiling of infection-causing pathogens remains a significant challenge in modern health care. Despite advances in molecular diagnostic techniques, blood culture analysis remains the gold standard for diagnosing sepsis. However, this method is too slow and cumbersome to significantly influence the initial management of patients. The swift initiation of precise and targeted antibiotic therapies depends on the ability of a sepsis diagnostic test to capture clinically relevant organisms along with antimicrobial resistance within 1 to 3 h. The administration of appropriate, narrow-spectrum antibiotics demands that such a test be extremely sensitive with a high negative predictive value. In addition, it should utilize small sample volumes and detect polymicrobial infections and contaminants. All of this must be accomplished with a platform that is easily integrated into the clinical workflow. In this review, we outline the limitations of routine blood culture testing

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and discuss how emerging sepsis technologies are converging on the characteristics of the ideal sepsis diagnostic test. We include seven molecular technologies that have been validated on clinical blood specimens or mock samples using human blood. In addition, we discuss advances in machine learning technologies that use electronic medical record data to provide contextual evaluation support for clinical decision-making.

KEYWORDS biomedical engineering, DNA sequencing, diagnostic, infectious disease, microbiology techniques

INTRODUCTION

Sepsis is a serious and life-threatening clinical condition that generally results from a primary bacterial infection or, less frequently, from a fungal and/or viral infection. Affecting nearly 1 out of every 23 hospitalized patients, it is the sixth most common reason for hospitalization (1–5). At present, it is the most expensive condition treated in U.S. hospitals, with an aggregate cost of US$15.4 billion in 2009 (4, 5), whereas nonspecific diagnoses of sepsis account for another US$23.7 billion each year (6, 7). Alarmingly, the incidence of sepsis is increasing, with a 17% increase in the number of documented cases between 2000 and 2010 (5), while sepsis-related deaths have surged 31% between 1999 and 2014 (8). Approximately 30,000 sepsis-related deaths occur annually, with particularly high rates in critically ill patients admitted to intensive care units (ICUs) (5, 9, 10).

Neonates, or infants within 28 days of life, comprise an additional group at risk for infection due to the relative deficiency of their adaptive immune responses from the lack of antigen exposure *in utero* as well as the immaturity of innate immune responses, impairments which are directly related to their gestational age at birth. In the United States, sepsis is the fifth leading cause of neonatal mortality, surpassed only by loss of life due to preterm birth and intrapartum complications (11–13). Furthermore, infection has been linked to preterm birth (14–16). Devastatingly, 25% of all neonates in the United States admitted to a neonatal ICU (NICU) will be diagnosed with sepsis, and 18 to 35% (21,000 neonates/year) will die from their infection (11, 17, 18). Low-birth-weight premature infants have a 10-fold increased risk of serious infection or sepsis compared to their full-term counterparts, with a 30% mortality rate (19–21).

Septic patients usually present with malaise, fever, chills, and leukocytosis, which often prompt care providers to evaluate patients for the presence of bacteria in the bloodstream (bacteremia) by using blood culture analysis. Considered a medical emergency, sepsis can rapidly progress to organ dysfunction and death despite immediate and aggressive medical therapies (10). In the absence of robust diagnostic tests, the reflexive utilization of broad-spectrum and highly potent antibiotic treatment in patients suspected of having sepsis has contributed to the emergence of drug-resistant organisms and atypical pathogens (22, 23). Survivors of sepsis may experience substantial long-term complications leading to a prolonged length of stay and/or discharge to a long-term-care setting (6). Neonatal sepsis survivors are at an increased risk for poor neurodevelopmental outcomes, including cerebral palsy, deafness, blindness, and cognitive delays (11, 24).

Because of the high mortality rate associated with sepsis, the dangers of undertreating some infections, or concerns about the use of inappropriate antibiotics, physicians tend to order blood cultures liberally (10). This results in bacteria being isolated in only 4 to 12% of processed blood culture tests. In more-restrictive settings where blood cultures are ordered less liberally, positivity rates can be much higher. Regardless, the detection of positivity occurs several hours to days after the patient has been treated (25–29).

Pathogen detection by blood culture is, unfortunately, worse in the neonatal patient population than in older children and adults. A major confounding factor arises from the fact that clinical signs related to sepsis are similar to those of other noninfectious life-threatening conditions, such as perinatal asphyxia, respiratory distress syndrome,

and symptoms associated with severe prematurity. Although more than 60% of sepsis evaluations occur in the first 3 days of life, <1% of blood culture tests detect an organism. Even in symptomatic neonates, blood culture methodologies can detect the offending microorganism in only 10 to 15% of neonates after contaminants are excluded (30, 31). This burden is worse in underserved communities. For example, in the United States, black preterm neonates have the highest incidence of and case fatality rate from neonatal sepsis (32). Around the world, neonates born in low- and middle-income countries suffer the highest rates of sepsis (33). Critically, in low- and middle-income countries, resistant bacterial strains are implicated in the majority of the cases, highlighting the need for rapid susceptibility testing.

Underrecognition of illness in addition to the emergence of resistant pathogens, delays in diagnosis, and the inability to access or afford specialized medical care contribute to the high mortality and morbidity rates associated with sepsis (34). The correct initial choice of antibiotic therapy has been shown to save more lives than any other medical intervention (35–38), and studies suggest that there is a 1- to 3-h diagnostic window, from symptom-based sepsis recognition to the initiation of antimicrobial treatment, before the mortality rate increases (39). The Surviving Sepsis campaign advocates for the implementation of antibiotic therapy within 1 h of clinical recognition of sepsis and collection of blood for culture prior to the administration of antibiotics (35). However, a 5-fold reduction in survival has been reported as a consequence of inappropriate antimicrobial therapy in the first 6 h after the recognition of sepsis (40). A recent editorial questioned blind antibiotic use and made a compelling case for the initiation of targeted antimicrobial therapy, preferably after the detection of the pathogen (41). Thus, rapid diagnostic tests that are capable of profiling antimicrobial resistance or ruling out bacterial infection as a cause of sepsis must be integrated into the initial 1 to 3 h of the clinical timeline to influence the appropriate use of antibiotics and patient outcomes.

Unfortunately, findings from standard diagnostic tests are not available within this critical time frame to allow focused, effective, and potentially life-saving medical interventions. Other faster adjunct standard hematological analyses used in routine clinical practice have low sensitivity and specificity, particularly in neonatal patients (42). Recently, biomarkers such as C-reactive protein (CRP), procalcitonin (PCT), and the neutrophil marker CD64 have made their way into sepsis evaluations, with limited success. Most diagnostic approaches that are currently employed rely on individual biomarkers, with binary yes or no answers. An integrative diagnostic strategy that incorporates a broader range of biomarkers could in theory characterize the host response to rule in/out infection, identify and quantify the pathogen(s), and predict resistance. Such a test is greatly needed to distinguish patients who are truly septic and assist with the appropriate use of antibiotics.

THE IDEAL SEPSIS DIAGNOSTIC TEST

Considering the current clinical challenges and the need to impact clinical management by informing targeted treatment, the ideal technology should include the following characteristics (43, 44):

1. rapid detection (the pathogen needs to be identified in less than 3 h) (35, 39);
2. broad-based detection, including bacteria, viruses, and fungi;
3. minimal invasiveness, utilizing clinical samples with low specimen volumes (<1 ml blood for pediatric patients, including neonates, and 5 to 10 ml blood for adults) (45–47);
4. high sensitivity and specificity for the immediate initiation of targeted antibiotic use in the presence of signs and symptoms of systemic inflammation (the diagnostic tests should not compromise on sensitivity with low pathogen levels in the specimen);
5. polymicrobial detection of pathogens in the presence of contaminants across a wide range of pathogen loads (~1 to 100,000 CFU/ml blood);

6. detection of drug resistance;
7. integration into the clinical workflow (the process should be easy to use and require minimal technical expertise to process samples and interpret test results; for the greatest impact, the technology must be usable in noncentralized low-resource settings);
8. the ability to detect unknown and emerging pathogens (detection capabilities must be able to easily expand without compromising the robustness of detection and the required specimen volume); and
9. the ability to distinguish the inflammatory response as being either host or pathogen driven (48, 49).

LIMITATIONS OF STANDARD DIAGNOSTIC BLOOD CULTURE METHODOLOGIES ("GOLD STANDARD")

Today, the use of standard culture techniques for the detection and isolation of pathogenic organisms from a sterile body fluid specimen is still considered the "gold standard" for the diagnosis of infection and sepsis (50). However, routine blood cultures can take 6 h to 5 days to grow an organism to detectable levels, with additional time being required to identify (24 h) and test for (48 h) antibiotic susceptibility (28, 51, 52). This test is also plagued by many complicating factors. First, the quantity of microbes present in circulation during bloodstream infection (BSI) is usually low, ranging from only 1 to 1×10^4 CFU/ml (24, 53–55). In older children and adults, routine blood culture tests are performed in timed sequences of up to four separate replicates comprising approximately 20 to 30 ml of blood each. This repeat blood sampling improves pathogen detection to capture the causative organism in 73 to 95% of cases (35, 55–58). Small sample volumes can therefore lead to false-negative results with conventional practices (59–61). Unfortunately, in neonates, especially very-low-birth-weight (VLBW) (birth weight of $<$1,500 g) premature infants, blood collection is restricted to a single sample with a minimal volume (1 ml), which can further hinder pathogen capture, particularly when the level of bacteremia is low (45–47). Neonatal sepsis concentrations often fall within the range of 1 to 1,000 CFU per ml, with some studies finding that concentrations in 68% of culture-positive cases fall below 10 CFU per ml (62, 63).

False-negative results can also occur due to the challenge of recovering infectious etiologies by routine blood culture techniques after the initiation of antibiotic therapy, which affects 28 to 63% of adults with suspected sepsis (35, 55, 61, 64, 65). Exposure to antimicrobials prior to blood culture testing is magnified in neonatal patients, as an estimated 30 to 35% of laboring women receive empirical intrapartum antibiotics for the prevention of neonatal group B *Streptococcus* (GBS) disease (21). Subsequently, compliance with Centers for Disease Control and Prevention (CDC) GBS guidelines exposes an estimated 65% of VLWB infants to antibiotics prior to birth (66–68). Prolonged delays in pathogen identification and antibiotic susceptibility testing also cause neonates to be unnecessarily exposed to broad-spectrum antibiotics, leading to bacterial antibiotic resistance in noninfected neonates while preventing targeted antimicrobial therapy in septic neonates. Additionally, prolonged broad-spectrum antibiotic exposure in neonates can lead to invasive fungal (*Candida*) infection, necrotizing enterocolitis, and death (17, 18, 69).

Failure to adhere to standard antiseptic procedures during sample collection can also lead to contaminated, or false-positive, blood culture results. In 2005, the College of American Pathologists reported an overall mean blood culture contamination rate of 2.89% in 356 institutions, with rates of 2.08% noted for neonatal patients and 2.92% noted for nonneonatal patients (70). Contamination rates for individual institutions in this study ranged from 2.15% to 3.67% and contributed to an additional estimated cost of US$5,506 per patient (70). Thus, contaminated samples can have enormous financial and clinical ramifications in adult populations in the United States, including 1,372 to 2,200 extra hospital days and an extra US$1.8 million to US$1.9 million in medical costs each year (71, 72). In pediatric patients, these tainted samples are associated with

readmission rates of 14 to 26% (61, 73, 74) and increased lengths of stay from 1 to 5.4 days (61, 72, 75). In low- and middle-income countries, where there is a dearth of trained medical staff and quality health care services, blood culture contamination is not uncommon and can have grave consequences. Notably, almost half of patients with false-positive blood cultures are treated with antimicrobials compared to those with true-positive test results (61, 76–78). Additionally, 40 to 50% of adult patients with bacteremia (and 70% with fungemia) received incorrect antimicrobial therapy during their empirical treatment period before microbiology culture results were available (1, 5, 79). This misuse of antimicrobial agents and delays in pathogen identification cause prolonged exposure to broad-spectrum antibiotics, which can also result in an increased number of *Clostridium difficile* infections, antibiotic allergic reactions and drug toxicity, antimicrobial-resistant bacterial strains, prolonged length of stay, and increased medical costs (5, 61, 80–82). Additional approaches to mitigate contamination have been described with some success, such as algorithms, including clinical judgments, numbers of positive blood culture sets among all sets obtained, and ancillary laboratory tests such as CRP and PCT measurements (83).

In summary, routinely used blood culture methods are not an ideal gold standard, as the results often come too late, are incomplete or not sensitive enough, and can be misleading and relatively labor-intensive. There is a crucial unmet need to shorten as well as improve current laboratory procedures for the detection and identification of microorganisms. In the last decade, various engineering innovations have generated promising pathogen detection approaches that incorporate sample preparation, molecular detection, automation, miniaturization, multiplexing, and high-throughput analysis toward the development of an effective diagnosis technology. The following sections give an overview of current and emerging detection systems designed for the rapid, sensitive, and cost-effective diagnosis of bloodstream infections.

TOWARDS DETECTION DIRECTLY FROM WHOLE BLOOD: CURRENT AND EMERGING TECHNOLOGIES FOR RAPID DIAGNOSIS OF MICROBIAL INFECTIONS WITHOUT CULTURE

In the United States today, nearly all U.S. Food and Drug Administration (FDA)-approved sepsis molecular diagnostic tests are postculture technologies, meaning that a blood sample must be cultured to allow the number of microbes to increase before the diagnostic test can be conducted. This initial growth-based amplification ensures sensitive detection but extends the diagnostic timeline such that test results do not effectively impact patient management. It also restricts the breadth of organisms detected by relying on a single culture medium formulation, which cannot support the growth of all organisms or may mask susceptibilities (84–87). While molecular diagnostic tests are completed within 20 min to 2 h, the initial step of blood culture takes several hours to days and may not be successful. Likewise, determining the antibiotic susceptibility of the pathogen also depends first on additional culture methods. Current technologies do not benefit antibiotic stewardship programs aimed at deescalating empirical antibiotic therapy and encouraging timely targeted treatment. Recent reviews by Opota et al. (55, 88), Kothari et al. (89), Afshari et al. (90), and Ecker et al. (91) describe the state of the art for such diagnostic tests in more detail. In this review, we focus on emerging technologies that are not dependent upon initial microbial growth. All technologies described in the following paragraphs are summarized in Table 1.

Modern Nucleic Acid Amplification Technologies

For several years, nucleic acid amplification technologies (NAATs) have promised to circumvent the need for bacterial growth. These technologies function by rapidly creating copies of DNA or RNA originating from pathogen or host cells through biochemical reactions, amplifying the nucleic acid sequences to a detectable level. The sequences are then used to identify the infecting agent or the status of the immune response. However, the promise of NAATs for revolutionizing sepsis diagnosis has yet to be realized. This can be attributed to challenges in reliably capturing and amplifying

TABLE 1 Emerging molecular diagnostics for pathogen detection from whole blood[a]

Assay (manufacturer)	Technique	Sample vol	Sample-to-result time (h)	Detection limit	% sensitivity/% specificity	Breadth of detection and ability to expand	Ability to detect polymicrobial infection	Antimicrobial resistance marker(s)
Iridica Plex ID (Abbott Molecular)	Multiplex broad-range PCR/ESI-MS	5 ml	6	0.25–128 CFU/ml	45–83/69–94	780 bacteria and *Candida*; highly expandable	Yes; semiquantification of load	*mecA*, *vanA*, *vanB*, and bla_{KPC}
SeptiFast (Roche Diagnostics)	Multiplex target-specific real-time PCR/*in situ* hybridization/melt analysis	1.5 ml	4–6	3–100 CFU/ml	63–83/83–95	>16 bacteria, *Candida*, and *Aspergillus fumigatus*; low expandability	Yes; semiquantification of load	*mecA* (after positive test for *Staphylococcus aureus*)
SepsiTest (Molzyme)	Universal PCR/sequencing	1 ml	8–10	10–80 CFU/ml	11–87/83–96	>345 bacteria and 13 fungi; highly expandable	Yes; no load quantification	None
MinION (Oxford Nanopore Technologies)	Nanopore sequencing	10 ng high-molecular-wt DNA (bacterium identification is not validated for whole blood)	4–6	~100 copies/ml	NA/97–100	Initial validation on few viruses and bacteria; highly expandable	Potentially yes with load quantification	Can be added in the future
U-dHRM	Digital PCR/high-resolution melt	1 ml	<4	Single cell	Single cell[b]/99.9	37 bacteria; highly expandable to include other bacteria, viruses, and fungi	Yes; absolute load quantification	Can be added in the future
SeptiCyte (Immunexpress)	RT-qPCR to quantify the host response of 4 RNA biomarkers; machine learning	2.5 ml	1–6	NA	NA/95 (able to discriminate SIRS from sepsis with an AUC of ~0.9)	All pathogens	NA	None
LAMP technology	Loop-mediated isothermal amplification	~30 μl to a few milliliters (varies for the specific LAMP technique used)	1	Single cell	Single cell[b]/100	No integrated platform for broad-based detection (1 pathogen per sample)	Limited	Can detect 1 resistance gene at a time in a separate sample
Integrated comprehensive droplet digital detection technology (IC 3D) (Velox Biosystems)	DNAzyme-based sensor droplet microencapsulation 3D particle counter system	Microliters to milliliters	1–4	Single cell	Single cell[b]	No integrated platform for broad-based detection (1 pathogen per sample)	Limited	Can be added in the future; limited by no. of fluorescence channels

[a]NA, not applicable.
[b]Preclinical studies.

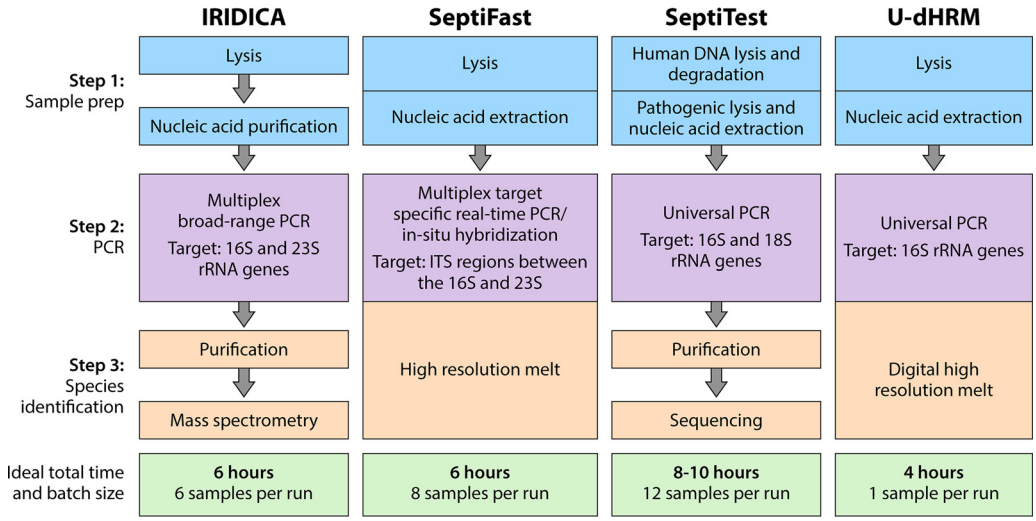

FIG 1 Workflow for the analysis of a single whole-blood specimen for pathogen identification. Even though U-dHRM shows promise as the fastest technology, it could benefit from parallelizing for multiple loads in the future.

pathogen nucleic acids from complex samples like blood, where the infecting agents are present at low levels or as polymicrobial mixtures within a high background of human DNA. In this sample context, traditional NAATs cannot simultaneously satisfy the need for sensitive, specific, and broad-based detection. The emerging technologies discussed here represent novel integrations of NAATs with other cutting-edge techniques that together are capable of overcoming many current diagnostic limitations. We also discuss the exciting promise that further synergistic integration holds for producing the ideal sepsis diagnostic test.

Iridica Plex ID. The Iridica Plex ID platform (Abbott Molecular, Des Plaines, IL) boasts the most broad-based detection of any direct-from-blood emerging technology, identifying an impressive 780 bacteria and *Candida* species with a turnaround time of 6 h (55, 92). However, only four antimicrobial resistance markers (*mecA*, *vanA*, *vanB*, and bla_{KPC}) are detected. Iridica accomplishes this by integrating multiplexed PCR amplification of pathogen DNA with electrospray ionization mass spectrometry (ESI-MS) for sequence identification. The process starts with automated DNA extraction from a 5-ml whole-blood sample. The extracted DNA is distributed across several PCR mixtures containing different primers targeting conserved regions of pathogen genomes, including the 16S and 23S rRNA genes for bacteria and *Candida*, respectively. These primers and reaction components have been optimized to limit interference due to human DNA, which can otherwise lead to nonspecific amplification or low amplification efficiency. Amplified copies from each reaction are selectively enriched by removing over 98% of human DNA. They are then assessed by ESI-MS, which generates nucleotide base composition data. Finally, the data from each amplicon are compared to a library of all expected base compositions and used to triangulate the pathogen species (Fig. 1) (54, 93).

While this approach achieves a wide breadth of detection, clinical studies show that the sensitivity, specificity, and negative predictive value (NPV) of Iridica vary widely from 45% to 83%, 69% to 94%, and 80% to 97%, respectively, against conventional culture methods (Table 2 and Fig. 2). After the exclusion of possible contaminant bacteria and estimation of true-positive rates based on PCR test replicates or clinical chart and culture results for patient-matched specimens, sensitivity and specificity values can be improved from 77% to 91% and from 87% to 99%, respectively (54, 92). Improvement with multiple test replicates suggests that sample heterogeneity and sampling error remain problematic. In the case of a low level of a pathogen, sampling error first occurs at the point of the blood draw, is combined with any inefficiency in nucleic acid extraction, and then occurs again when the sample is split across multiple

TABLE 2 Characteristics of studies reviewed for data on the Iridica platform

Reference	Patient setting(s)	No. of paired tests	Blood vol (ml)	Inclusion criterion	% sensitivity[a]	% specificity[a]	NPV (%)[a]
54	ICU	331	5	Suspected bloodstream infection	83	93.6	98.9
95	ICU	616	5	Suspected or proven sepsis or severe infection	81	69	97
92	ICU, ER	408	5	Suspected sepsis	74.8	78.6	74.1
	ICU	220	5		78.4	70.8	95
93	ER	285	5	≥2 SIRS criteria for sepsis	83	72	94
94	Hematology	105	5	Febrile neutropenia	45	93	80

[a]Against blood culture.

distinct amplification reactions targeting different genes on the Iridica platform. Improving the sensitivity and reliability of detection will require circumventing these sources of error. For the same reasons plus amplification competition, polymicrobial samples may present another challenge for this approach. Some evidence suggests that the Iridica platform can detect mixed pathogen populations, but its utility for clinical samples is currently inconclusive. We found only one study that investigated polymicrobial specimens. Here, the Iridica platform identified only one causative organism in four out of nine cases of blood culture-positive polymicrobial infection (94).

Iridica has been evaluated in a limited number of clinical studies across patients with suspected sepsis, systematic inflammatory respiratory syndrome (SIRS), and febrile neutropenia (54, 92–95). Interestingly, significant differences in sensitivity have been reported across ICU and emergency room (ER) patients ($P = 0.005$), with higher sensitivity being seen for ICU patients (92). This may derive from higher pathogen loads in this patient population, which would have the effect of reducing sampling error. Limits of detection of the Iridica platform range from 0.25 to 128 CFU/ml for bacteria, depending on the target species, and 4 CFU/ml for *Candida* species (54, 93).

This broad-based semiquantitative technology shows promise for use on whole-blood samples to detect a wide variety of pathogens, but its potential impact on antibiotic stewardship is low given the limited number of resistance markers detected. However, this technology benefits from the ability to expand the test to more targets in the future. The use of 5 ml blood is promising for adult patients but limits feasibility for use on pediatric patients (96). The Iridica platform is an end-to-end diagnostic solution with a structured and easy-to-use workflow. Individual steps are automated, thus reducing labor and increasing efficiency. The time to detection ranges from

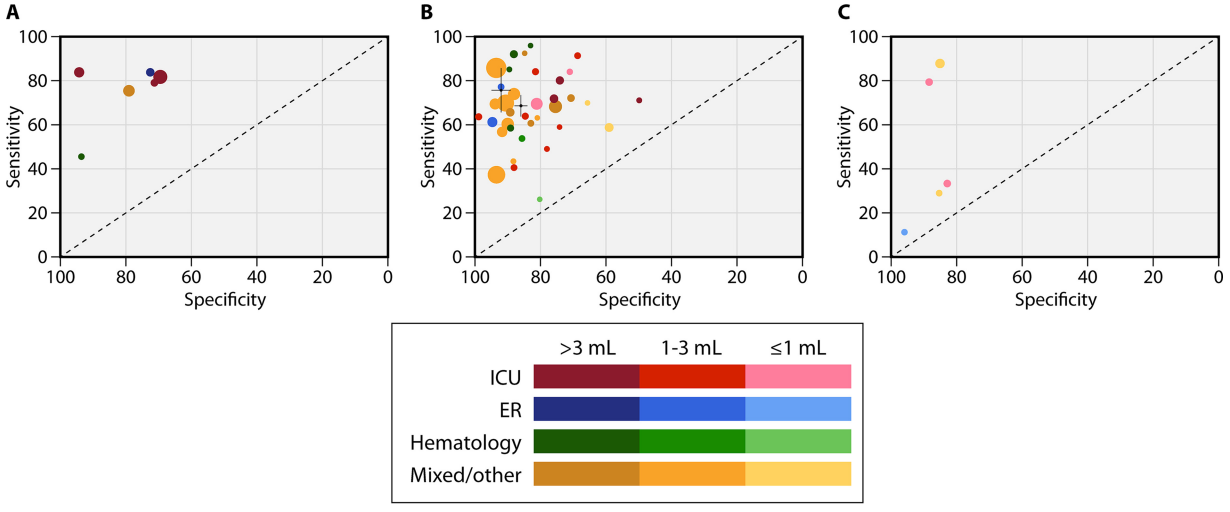

FIG 2 Sensitivity plotted against specificity of test results compared against the gold standard of blood culture for Iridica, SeptiFast, and SepsiTest. The marker/symbol area is proportional to the number of paired blood tests in the study. Darker shades of color signify larger blood volumes used for the test. (A) For Iridica, we included data from 6 publications found by a PubMed literature search. (B) For SeptiFast, we included data from 2 meta-analyses (summary statistics from analyses are shown in black, along with the confidence intervals) in addition to data from 8 new relevant studies. (C) For SepsiTest, we included data from 5 publications found by a PubMed literature search.

between 6 and 8 h with only 30 min of hands-on time for a batch of 6 samples (93, 94). However, this technology fails to meet the ideal turnaround time of 1 to 3 h. This technology is not yet approved by the U.S. FDA but is Conformité Européenne (CE) marked, meaning that it complies with the European In-Vitro Diagnostic Devices Directive and is commercially available in Europe (55, 91). However, it may fall short in noncentralized clinical settings due to the dependence on multiple bulky devices and high up-front costs of about US$357,043 (90, 97). The cost per test ranges from US$262.92 to US$419.14 (assuming that £1 equals US$1.33) (98).

SeptiFast. SeptiFast (Roche Diagnostics, Risch-Rotkreuz, Switzerland) is a commercially available (in the European Union), broad-based microbe identification test for whole blood. It can identify over 16 bacteria, 5 *Candida* species, and *Aspergillus fumigatus* fungi using a 1.5-ml whole-blood sample within 6 h. In addition, it can detect the *mecA* antibiotic resistance gene after a sample tests positive for *Staphylococcus aureus*. This technology is CE marked but not yet FDA approved.

The SeptiFast test integrates multiplexed real-time PCR with probe hybridization and DNA melting analysis. The test begins with nucleic acid extraction from whole blood under a contamination-controlled workflow. This is followed by real-time PCR amplification using a combination of universal and specific primers in three parallel reactions for Gram-positive bacteria, Gram-negative bacteria, and fungi (99). The primers target the internal transcribed spacer (ITS) regions between the 16S and 23S genes for bacteria and between the 18S and 5.8S genes for fungi. PCR products are detected by using species-specific probes that fluoresce in one of the four detection channels. Species identified in the same detection channel are subsequently differentiated by using melting temperature analysis (Fig. 1) (99, 100).

SeptiFast has a reported sensitivity of between 3 and 100 CFU per ml, depending on the microorganism (99). A meta-analysis of 41 studies reported a summary sensitivity and specificity of 68% (95% confidence interval [CI], 63% to 73%) and 86% (95% CI, 84% to 89%), respectively, for a total of 10,493 SeptiFast tests compared to blood culture (101). Another meta-analysis that included only data from journal publications reported slightly better overall sensitivity and specificity, 75% (95% CI, 65% to 83%) and 92% (95% CI, 90% to 95%), respectively, based on 8,438 tests (102). Recent studies show similar heterogeneous results (Table 3 and Fig. 2) (100, 103–140). These numbers improved when studies incorporated clinical markers along with blood culture results (124, 125, 137, 141). However, as many as 35% of the SeptiFast-positive episodes were not supported by any microbiological or clinical data (109, 120). On the other hand, low sensitivity prevented SeptiFast from identifying culture-positive organisms in 20 to 30% of cases (142). SeptiFast has been reported to resolve polymicrobial infections with higher detection rates ($\chi^2 = 4.50$; $P = 0.0339$) than blood culture (121, 132, 133, 143, 144). However, the detection of mixed pathogens may be hindered by competing amplification due to the use of multiple specific primers and needs further investigation (137).

In summary, SeptiFast may be considered broad-based, with coverage of the 25 most relevant pathogens for sepsis, and incorporates the ability to detect mixed pathogen populations. However, it is missing pathogens that are highly relevant for neonatal sepsis. This technology considerably lowers the blood volume needed for testing compared to that needed for conventional technologies, which could be beneficial for pediatric patients (100). However, 1.5 ml of blood is excessive for neonates, for whom samples are limited to 1 ml. SeptiFast, when used with MagNA Pure (Roche) automated DNA extraction, shortens the complete workflow time to 3.57 h for eight parallel loads (115). This diagnostic test may be of added value for the management of patients with suspected sepsis who are SeptiFast positive but blood culture negative (106, 121, 129, 145, 146). However, low sensitivity deems negative results nonactionable. It may also fall short in noncentralized clinical settings due to the dependence on multiple bulky devices and up-front costs of about US$35,167 (90, 97). The cost per test is estimated to range from US$204.73 to US$273.83 (assuming that £1 equals US$1.33) (98). Other limitations include incomplete antibiotic resistance infor-

TABLE 3 Characteristics of studies reviewed for data on SeptiFast[a]

Reference	Patient setting(s)	No. of paired tests	Blood vol	Inclusion criterion(s)	% sensitivity[b]	% specificity[b]	NPV (%)[b]
103	ICU, ER, other hospital admits	200	3 ml	Suspected BSI with ≥2 SIRS criteria	65.1	89.2	90.3
104	Hematology	103	1.5 ml	Febrile oncohematological patients	95.2	83	98.6
105	ICU, hematology, other hospital admits	36	NA	Suspected sepsis with empirical antibiotic treatment	50	71	84.6
106	ICU, surgery, other hospital admits	101	3 ml	Suspected sepsis	60.1	82.9	87.5
107	Hematology/oncology	134	1 ml	Febrile neutropenia after chemotherapy	25.6	80.2	65.5
108	Neonatal ICU	34	1.5 ml	Suspected late-onset sepsis	75	86.7	96.3
108	Hematology	154	1.5 or 3 ml (wt of ≤45 kg or >45 kg)	Fever in immunocompromised patients	57.9	89.1	83.7
109	NA	558	5 ml	NA	67.6	75.5	94.1
110	ER	144	1.5 ml	Suspected BSI and ≥2 SIRS criteria	69.7	90.1	87.5
111	ICU	236	3 ml	Suspected sepsis	79.5	74.1	94.8
112	ICU	453	1 ml	Clinical suspicion of severe sepsis	68.9	81.2	94.6
113	Hematology/oncology	110	1.5 ml	Suspected sepsis	53.1	85.6	81.7
114	ER, ICU	78	3 ml	Suspected BSI and ≥2 SIRS criteria	91.7	84.8	98.2
115	ICU	106	1.5 ml	SIRS and suspected sepsis	63	98.7	88.6
116	ER	306	1.5 ml	Suspected infection or ≥2 signs of SIRS	60.6	94.6	89.7
117	ICU	100	1.5 ml	Fever or hypothermia	40	88	93.1
118	ER, ICU, hematology, others	400	1.5 ml	Suspected SIRS	68.7	93.7	97.2
119	ICU	86	1.5 ml	Febrile neutropenia	48.4	78	66.7
120	Hospital admits	1,141	1.5 ml	Suspected sepsis	36.8	93.3	91.2
100	ER, ICU, hematology	1,553	>1.5 ml	Suspected sepsis in neonates and children	85	93.5	98.1
122	ICU, hematology/oncology, other hospital admits	71	1.5 ml	Suspected sepsis	62.5	80.9	94.4
123	Hematology	166	1.5 ml	Febrile neutropenia	91.3	88.1	98.4
124	ER	119	1.5 ml	Suspected sepsis	76.4	91.9	82.6
121	ICU	148	1 ml	SIRS	83.3	71.1	94.4
125	Hematology/oncology	79	1.5 ml	Suspected sepsis in immunocompromised patients	84.4	89.4	89.3
126	Hospital admits	391	1.5 ml	Suspected sepsis	56.1	91.6	92.4
127	ICU	225	3 ml	Suspected sepsis after liver transplant	71.2	75.9	82.7
128	ICU	110	1.5 ml	Suspected sepsis	63.2	84.6	91.7
129	ICU, other hospital admits	160	1.5–3 ml	Suspected sepsis or septic shock	71.4	70.7	89.2
130	Critically ill	75	NA	NA	42.8	88.2	93.8
131	ER, ICU	525	1.5 ml	Suspected BSI and ≥2 SIRS criteria	59.7	89.9	86.4
132	ICU, ER, other hospital admits	1,186	1.5 ml	SIRS	69.2	90.6	94.1
133	Hospital admits	86	1 ml	Clinical sepsis suspicion and those presenting a score of >8 points on the NOSEP-1 scale[c] in neonates	69.2	65.7	92.3
134	ICU	138	1.5 ml	Bacteremia with empirical antibiotic	83.3	81.5	94.6
135	ICU	78	1.5 ml	Suspected pulmonary or abdominal sepsis	58.3	74.2	90.7
136	ICU	78	3 ml	Suspected or diagnosed sepsis or septic shock	70.4	50	80.4
137	Surgery, other hospital admits	220	1.5 ml	≥2 SIRS criteria	69.4	89.7	91.2
138	ICU	119	1.5 ml	Severe sepsis or septic shock	90.6	68.7	94.8
139	Hospital admits	214	100 μl	Late-onset sepsis in VLBW neonates	58.1	59.1	80.3
140	ICU, ER, outpatient	470	NA	Sepsis, endocarditis, fever, pneumonia, and immunosuppression	73.3	88	95.3

[a]The PCR test was compared to blood culture by identified organism. If data by organism were not available, comparisons were made by samples. ICU, intensive care unit; ER, emergency room; SIRS, systemic inflammatory response syndrome; NPV, negative predictive value.

[b]Against blood culture.

[c]The NOSEP (nosocomial sepsis)-1 scale is composed of C-reactive protein levels of >14 mg/dl (5 points), neutrophil counts of >50% (3 points), thrombocytopenia level of $<150 \times 10^6$/liter (5 points), parenteral nutrition for >14 days (6 points), and fever of >38.2°C (5 points).

TABLE 4 Characteristics of studies reviewed for data on SepsiTest[a]

Reference	Patient setting(s)	No. of paired tests	Blood vol (ml)	Inclusion criterion(s)	% sensitivity[b]	% specificity[b]	NPV (%)[b]
152	ICU, hematology/oncology	342	1	SIRS or sepsis, hematology/oncology with febrile neutropenia, or immunodeficiency and fever	87	85.2	97.2
151	Surgery IE	30	1	Infectious endocarditis	85	NA	NA
130	Critical care	75	NA	NA	28.6	85.3	92
150	ER	125	1	≥2 SIRS criteria for sepsis	11	96	80
154	ICU	160	1	High-risk patients on ECMO	78.6	88.4	97.7
153	ICU	236	1	SIRS or suspected sepsis	33	82.9	84.7

[a]IE, infectious endocarditis; ECMO, extracorporeal membrane oxygenation.
[b]Against blood culture.

mation and the inability to expand the test due to a limited number of detection channels.

SepsiTest. SepsiTest (Molzyme, Bremen, Germany) is a commercially available (in the European Union) broad-based microbial identification test for whole blood. It can identify over 345 bacteria and 13 fungi in 8 to 10 h from a 1-ml whole-blood sample. This technology is CE marked and commercially available in Europe but not yet FDA approved.

SepsiTest integrates universal PCR with Sanger sequencing after a unique sample preparation step whereby selective lysis and human DNA degradation are used to improve sensitivity (147). After DNA is isolated, PCR is performed with a universal primer targeting the 16S and 18S rRNA genes for bacteria and fungi, respectively. Bacteremia or fungemia is reported in <4 h. Further purification followed by Sanger sequencing accomplishes species detection, which takes an additional 4 to 6 h (Fig. 1).

SepsiTest can detect as few as 10 to 80 CFU/ml, with some organism bias (148, 149). It has a reported sensitivity ranging from 11% to 87% and a high specificity ranging from 85% to 96% compared to blood culture in adult and pediatric patients with SIRS, sepsis, febrile neutropenia, and infectious endocarditis (130, 150–154) (Table 4 and Fig. 2). Multiple studies report promising NPVs close to 97% against blood culture with the detection of multiple fastidious organisms (152, 154). Similar sensitivities ranging from 37.5% to 78.6% and specificities ranging from 86.8% to 94.4% were observed in studies adjusting for clinical context by excluding contaminants (130, 153). Additionally, as many as 45% of PCR-positive test results were reported to be due to contaminants (153). Pathogens detected in mixed populations were often identified as contaminants (151, 152). In one reported study, only one organism was identified in three of four blood culture-positive polymicrobial specimens (152).

SepsiTest is a broad-based test that requires a small amount of blood appropriate for both adult and pediatric patients. It can, in principle, detect polymicrobial infections; however, its ability to inform clinical decision-making needs further study. SepsiTest provides the option to automate DNA extraction (SelectNA plus; Molzyme) and process up to 12 samples in one run, making it easy to integrate into the clinical workflow. However, it does not provide any information on antibiotic sensitivity. In addition, it still requires multiple steps that are not integrated into one platform, increasing the risk of contamination and the turnaround time. This limits its utility for informing clinical decisions regarding targeted antimicrobial therapy. It may also fall short in noncentralized clinical settings due to the dependence on multiple bulky devices and high up-front costs of about US$57,553 (90, 97). The cost per test ranges from US$144.28 to US$199.21 (assuming that £1 equals US$1.33) (98). The use of Sanger sequencing is the time-limiting step for SepsiTest. In the future, massively parallelized next-generation sequencing technologies may enhance this approach and provide antibiotic resistance information. In the next paragraph, we provide a short summary of such an emerging sequencing technology.

Nanopore sequencing (MinION). The MinION (Oxford Nanopore Technologies, Oxford, United Kingdom) is a portable, real-time, USB-powered DNA/RNA sequencer

with a 10- to 50-min library preparation step. The main advantages of the MinION platform over other next-generation sequencing technologies are its (i) rapid turnaround, (ii) low capital cost, and (iii) small size. This technology was released to researchers for alpha testing as part of an early-access program in 2014 (155). It is a generic sequencing system that has shown the potential for the rapid identification of pathogens (<4 h) (156) directly from blood when combined with a PCR amplification step using the 16S Rapid Amplicon Sequencing kit (155). Because it performs sequencing at the single-molecule level, it offers new possibilities to study microbial diversity in clinical samples and also allows multiplexing of samples. This technology has been validated for viral pathogen identification from 140 μl whole blood in <40 min with 100% sensitivity and specificity (157, 158). For bacteria, it has been validated only with clinical urine and fecal samples (156, 158). Polymicrobial pathogen identification has been demonstrated by using genomic DNA mixtures of 20 bacterial strains in equal amounts (100,000 copies per organism per μl) (159, 160). By using specific primers that amplify a wide range of bacterial 16S rRNA genes, 90% of the full-length 16S rRNA gene could be reconstructed with the MinION nanopore technology. However, pathogen assignment could be completed for only 8 of the pathogens from the DNA mixture due to low sequencing coverage. This was attributed to nonoptimized 16S PCR amplification, despite the use of universal primers (159). This points to the need for the optimization and validation of this technology as a complete system with whole blood. Other improvements are needed to transition MinION into the clinic. These improvements include automation; standardized external and internal spike-in controls that run in parallel to prevent carryover contamination (161); as well as optimization of the bioinformatic pipeline used to identify organisms, resistance genes, and/or mutations (156, 162, 163). A major benefit of this technology is that it does not require up-front capital costs. According to the Oxford Nanopore Technology website, it is currently being offered to members of a developer-style access program for US$1,000, which includes the MinION device, three flow cells, two reagent kits, and software.

U-dHRM and machine learning on pathogen DNA fingerprints. The universal digital high-resolution melt (U-dHRM) platform is a broad-based microbial identification technology used with whole-blood samples. It can currently detect 37 bacterial pathogens with single-organism and single-genome sensitivity as well as resolve polymicrobial infections in <4 h using <1 ml whole blood (164, 165). This technology is in the validation phase (University of California, San Diego) and is not yet commercially available.

U-dHRM integrates universal digital PCR (dPCR) with high-resolution melt (HRM) analysis on a microfluidic chip to enable probe-free differentiation and quantification of bacteria within a sample (165). The test procedure begins with DNA extraction followed by sample "digitization," which separates all pathogen genomes into their own PCR mixtures by spreading the sample across a microfluidic chip containing 20,000 picoliter-sized reaction mixtures. In each reaction, universal amplification targeting the 16S rRNA gene takes place. Subsequently, precise heating and simultaneous imaging are performed on all reaction mixtures to generate HRM curve fingerprints for each pathogen's 16S gene sequence (Fig. 1). HRM generates sequence-specific melt curves by unwinding DNA amplicons in the presence of a fluorescent double-stranded intercalating dye (166–169). Each distinct DNA sequence melts uniquely, generating a loss of the fluorescence signature as a function of temperature that is then used for species identification (Fig. 3). A supervised machine learning algorithm automatically identifies the microbial species by its melt curve. U-dHRM has reported a classification accuracy of 99.9% for the 37 pathogens tested, with load quantification for individual pathogens (165). This technology was validated by using mock blood samples, demonstrating its ability to identify pathogens in the presence of excessive human DNA (165).

U-dHRM is a rapid, broad-based test to detect multiple organisms in a blood sample of less than 1 ml, which is suited for pediatric patients and neonates. While it is currently limited to 37 bacteria relevant to neonatal sepsis, it has the potential to expand to include additional bacteria, fungi, and viruses in the future. Since this

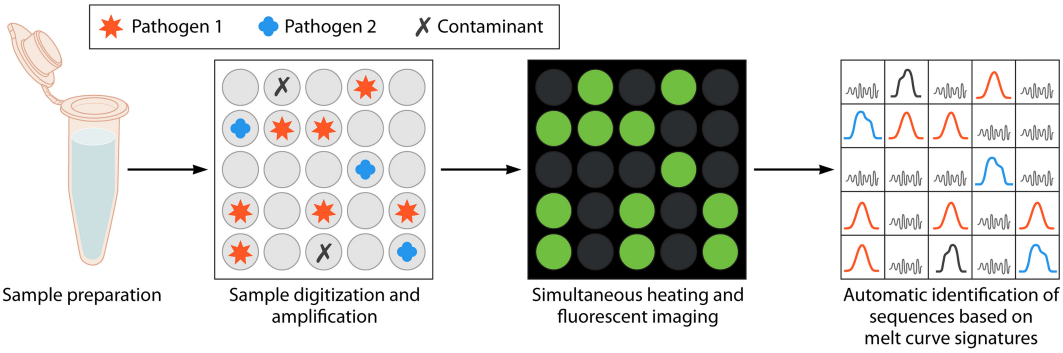

FIG 3 Digitization and melting of genomes after amplification with PCR technology. A melt curve corresponding to the individual genome is generated for identification and absolute load quantification of the pathogen and contaminants.

technology is probe free and digitized, it has the potential to detect all sepsis-causing organisms contained in a single sample, including polymicrobial infections. Early studies showed promising single-genome sensitivity and 99.9% specificity, but further evaluation with clinical blood samples is needed. Validation at other sites will also be important. In addition, automation is required to accomplish a sample-to-answer time of <3 h. This system is easy to use and can incorporate the detection of antibiotic resistance determinants. Its machine learning framework provides the potential identification of new and unknown pathogens and allows for an expanding library. The speed and simplicity of U-dHRM along with its integrated technology platform suggest a promising first-pass screening method for neonatal sepsis. This technology also shows the potential to deliver at- or near-point-of-care diagnosis. The possibility of moving U-dHRM toward a portable, inexpensive system can be of immense value to noncentralized systems in low-resource settings (170, 171), where the cost per test must typically fall between US$1 and US$30.

Summary of modern nucleic acid amplification technologies. In summary, the results of clinical studies using PCR-based technologies are heterogeneous. For the most part, these results are reported in comparison with the gold standard of blood culture, which is far from ideal and may contribute significantly to this heterogeneity. Blood may be drawn with varied timing, at different bodily locations, and in various amounts for blood culture. This contributes to the challenge of validating emerging technologies against blood culture. Hence, it is important to interpret diagnostic results in conjunction with clinical context.

(i) Interpreting false-positive results against blood culture. One of the major advantages of a PCR-based technology is its ability to detect nonviable, fastidious, and unculturable organisms that would otherwise be missed by blood culture. A PCR-positive, blood culture-negative specimen may reflect a real pathogen yet leads to biased lower sensitivity and specificity values of the PCR test. Hence, it is crucial that we carefully design comparison studies to include other molecular and adjunct tests instead of relying solely on an imperfect gold standard. It should be noted that false-positive results could also be due to cell-free pathogen DNA circulating in the blood originating from an old or controlled infection or contamination (172). Typically, PCR-based methods are unable to differentiate between viable and nonviable DNA (173). On the contrary, the use of 16S gene primers to amplify long 1-kb amplicons by U-dHRM may allow the differentiation of intact DNA relevant to active infection from the degraded DNA of dead pathogens or from the environment (165, 174). This can be a significant advantage over other molecular diagnostic tests, including sequencing technologies, which detect a high background level of organisms; clinical trials are pending. It has been reported that both Iridica and SepsiTest have higher rates of contamination than blood culture (95, 153), which likely arises from the use of broad-based universal primers that are targeted to amplify short fragments of DNA. In

addition, as shown in Fig. 2, SepsiTest and Iridica involve more sample transfer steps than SeptiFast and U-dHRM. This further increases the risk of contamination (153).

Both SeptiFast and Iridica use semiquantitative methods to detect contaminants and limit false-positive results. SeptiFast uses a cutoff value that represents the number of PCR cycles at which DNA is adequately amplified to identify contaminants (175, 176). Iridica also uses similar thresholds based on the number of genomes per well to limit contaminants and reduce false-positive results. However, these techniques may need further optimization, as they can conversely lead to false-negative results (92, 176). Absolute load quantification in conjunction with clinical characteristics may improve diagnostic accuracy as well (175). An emerging theme is the need for integrating quantitative results with clinical context, potentially provided by a machine learning framework. For example, a diagnostic algorithm that uses a patient's CD64 index to determine whether SepsiTest should be performed has been proposed. This approach showed an improved detection of pathogens in patients with suspected BSI (177). A similar approach in conjunction with neutrophil/lymphocyte count ratios and levels of presepsin and procalcitonin has been suggested (150, 178).

U-dHRM manages contamination through the use of small reaction mixture volumes, which keeps contaminants from overwhelming low-level pathogen DNA in the amplification step. This also enables absolute quantification, since each organism's genome is amplified individually, without affecting detection sensitivity (164). It also integrates the amplification (dPCR) and detection (HRM) steps into a single closed system, which eliminates contamination due to sample transfer and reduces hands-on time (164). Importantly, the ease of use, speed, and quantitative power of this technology could enable repeated testing to track the appearance and removal of bacterial DNA in the blood during antibiotic treatment. In combination with host inflammatory markers, such repeated testing could lend deeper insights into the progression of sepsis. Having the ability to conduct repeated testing over time could reveal novel disease dynamics that may contribute to a further understanding of pathogen detection inconsistencies that often arise in technology comparison studies. U-dHRM also holds promise to address the need for point-of-care diagnostic tests, whereas other commercially available PCR tests typically need bulky and expensive equipment that is not feasible for use in noncentralized systems.

(ii) Interpreting false-negative results against blood culture. While false-positive results may lead to the inaccurate overuse of antibiotics and contribute to the generation of resistant organisms, false-negative results and the inaccurate withholding of antibiotic treatment are more immediately threatening to patient welfare (179). Accurately withholding empirical antibiotic use will require an improved sensitivity of PCR technologies (>98% negative predictive value) (43). PCR tests can be limited in their ability to detect pathogens for a variety of reasons, including the need for effective lysis across a broad range of microbes, the interference of human DNA or other inhibitory substances carried over from blood into the assay mixtures, the effect of off-target interactions, and amplification bias (180–182). It is interesting to note that even though all the above-described technologies rely on an initial PCR amplification step for microbe detection followed by a secondary step for species identification, they differ in their diagnostic sensitivities. The two major contributors to these differences are (i) the approach used for reducing interference from human DNA and (ii) the amplification strategy using either a single universal primer (SeptiTest and U-dHRM) or multiple broad-range or species-specific primers (Iridica and SeptiFast), which may suffer from amplification competition (Fig. 1). All commercially available PCR tests have optimized their workflow to improve pathogen DNA amplification, yet none of them show promise to replace blood culture due to their limited sensitivity for clinical specimens (Fig. 2). The Iridica platform recently increased the sample volume 5-fold, from 1 ml to 5 ml, under the assumption that low sensitivity arises from the inefficient capture of the pathogen in low-volume blood samples (54).

The enhanced sensitivity of U-dHRM is attributed to the diluting effect of the digital reaction format on inhibitory substances and the optimized dPCR reaction conditions

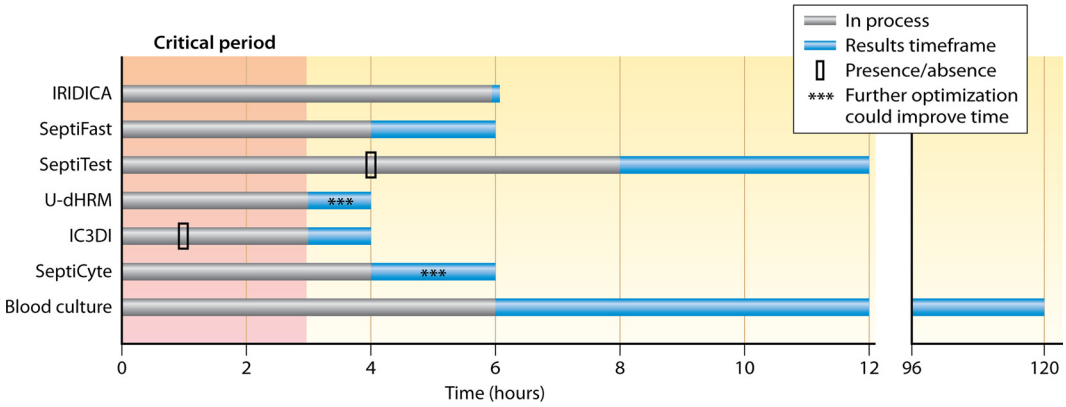

FIG 4 Timeline of sepsis technologies and where they fall compared to the gold standard of blood culture and the 1- to 3-h critical time for affecting clinical decision-making. SeptiCyte and U-dHRM may be further optimized to provide results in a shorter time frame.

ensuring the amplification of single copies of bacterial DNA. U-dHRM has been shown to significantly reduce false-negative error rates compared to traditional dPCR, indicating that amplification errors can be reliably identified and accounted for (164). In addition, U-dHRM is the only test that provides absolute load quantification to enable the resolution of polymicrobial infections and contamination. Further investigation with clinical samples will determine how this approach compares with commercially available technologies.

(iii) Turnaround times. The ideal turnaround times for all the technologies reviewed here are summarized in Fig. 4. The advertised turnaround times of 6 to 8 h for commercially available technologies, including Iridica, SeptiFast, and SepsiTest, may be optimistic for noncentralized and low-resources settings, where the sample-to-result time can be up to 16 h (134, 135). This increased time represents the time for batchwise analysis, sample transfer, and availability of staff. With the possibility of integration as near-point-of-care diagnostic tests, the estimates of $<$4-h turnaround times for MinION and U-dHRM are more realistic. Here, it is also worth briefly mentioning loop-mediated isothermal amplification (LAMP) technologies, as they offer high portability and short turnaround times. Importantly, however, they cannot be expanded to include a broad range of organisms without losses in sensitivity and specificity (183). The use of multiple specific primers does not allow multiplexing for a large number of pathogens. Alternatively, parallelizing tests using a small blood volume is also undesirable, as it results in a loss of sensitivity for the detection of low-level pathogen loads often associated with sepsis. That being said, LAMP-based tests may be suitable for applications where the detection of a single organism or class of pathogens is desired. The features of isothermal amplification and low technical complexity are particularly advantageous for low-resources settings (184, 185) and could support diagnosis for specific clinical presentations such as tuberculosis or malaria. Likewise, biosurveillance testing for specific viruses could also benefit from the LAMP approach. However, this method is unlikely to replace blood culture in clinics for general infection screening.

Host-Targeted Technologies

SeptiCyte Lab. SeptiCyte Lab (Immunexpress Inc., Seattle, WA) is the first RNA-based technology that targets specific human inflammatory markers using 2.5 ml whole blood for sepsis determination in 4 to 6 h. It has 510(k) clearance from the U.S. FDA for use as an aid in differentiating infection-positive (sepsis) from infection-negative systemic inflammation (SIRS) in critically ill patients on their first day of ICU admission.

SeptiCyte Lab is a host response-targeted, reverse transcription-quantitative PCR (RT-qPCR)-based test that quantifies the relative expression levels of four RNA biomarkers (CEACAM4, LAMP1, PLA2G7, and PLAC8) known to be involved in innate immunity and the host response to infection. In the discovery phase, microarray analysis was used

to identify RNA biomarkers that could differentiate patients with sepsis from patients with postsurgical infection-negative systemic inflammation (186). These biomarkers were then converted to a RT-qPCR format and used to develop the SeptiCyte Lab test for sepsis (186). SeptiCyte Lab is rapid, robust, and accurate for classifying patients with infection-related sepsis across gender, race, age, and date of ICU admission (186). It has been suggested to be an indicator of the probability and not the severity of sepsis (187, 188). In a pilot study using 2.5 ml of blood, SeptiCyte Lab effectively discriminated between two groups of critically ill pediatric patients (40 children with clinical severe sepsis syndrome versus 30 children with congenital heart disease). The area under the curve (AUC) in receiver operating characteristic (ROC) curve analysis, which describes the probability that a test will rank a positive incident higher than a negative one when chosen at random, was used to discriminate between the two cohorts. Even for different RNA analysis techniques, an AUC value of $>$0.9 was obtained (0.99 versus 0.95), indicating high accuracy. In another prospective observational study with 129 adult ICU patients, an AUC of 0.88 was obtained to discriminate SIRS from sepsis. SeptiCyte Lab scores have shown the ability to classify sepsis better than individual or combinations of other clinical, demographic, and laboratory markers (189).

SeptiCyte is a promising, novel, broad-based diagnostic test for sepsis. The current 4- to 6-h turnaround time can potentially be reduced to a targeted 1.5 h by optimizing the RT-qPCR platform on which the test is implemented. One drawback is the requirement for 2.5 ml of blood, which is not feasible for use on neonatal populations. Additionally, this test does not provide any information about the pathogen or its antibiotic resistance. However, a determination of infection-negative SIRS could serve to limit antibiotic treatment. More clinical studies across different patient populations are needed to confirm the ability of SeptiCyte to improve outcomes in the clinic. Nonetheless, it has the potential to play a role in reducing inappropriate empirical antibiotic use, which could be of tremendous value in light of the recent antibiotic resistance epidemic. This technology has not yet commercially launched, and no cost information is available at this time. Combining SeptiCyte with pathogen- and resistance-targeted tests that work within the same critical time frame could generate significant synergy, with the potential to enhance the overall NPV of these diagnostic tests and their impact on antibiotic use. Furthermore, such combined approaches may deepen our understanding of the progression of infection-related sepsis.

Amplification-Free Technology

Droplet digital detection technology. An emerging technology termed "integrated comprehensive droplet digital detection" (IC 3D) (Velox Biosystems, Irvine, CA) claims to selectively detect individual bacterial species directly from small quantities of whole blood within 1 to 4 h (190). In a one-step, culture- and amplification-free process, the IC 3D method provides quantitative bacterial detection with single-cell sensitivity.

IC 3D combines DNAzyme-based sensors with real-time droplet microencapsulation and a particle counter. First, blood samples are directly partitioned into billions of micrometer-sized droplets containing bacteria and a solution containing a fluorescent DNA sensor. The sensor is a DNA probe conjugated to a fluorescent reporter. Upon hybridization to the target sequence, the probe is cleaved and generates a fluorescent signal. Thus, droplets containing bacterial genetic material can be identified by fluorescence. A three-dimensional particle counter is then used to rapidly, robustly, and accurately quantify the fluorescent droplets containing bacteria (191, 192). The distribution of the blood sample into many small droplets minimizes interference from components of blood, making it possible to directly detect target bacteria without sample preparation and purification. In a proof-of-concept study, where blood was infused with *Escherichia coli*, the IC 3D method confirmed the presence or absence of this target bacterium within an hour. Quantitative measurement of the amount of *E. coli* bacteria was accomplished in about 3.5 h. In samples containing 1 cell per ml, the assay detected bacteria about 77% of the time (190).

This technology accomplishes rapid pathogen detection with a small blood volume

with single-cell sensitivity in a relatively easy-to-use format. Additional probes could be added to detect antibiotic resistance markers. However, the current system design is limited by its ability to detect only one bacterial species (e.g., *E. coli*) per analysis. There is the potential to expand the sensor set and develop a multiple-wavelength detection system for the detection of multiple bacteria or pathogens (190). However, the extent of this expansion would be limited by the small number of fluorescent channels and would not be able to incorporate the detection of emerging pathogens. Furthermore, the specificity of this technology has not yet been determined, and this technique has not yet been validated by using clinical samples. This technology is currently under commercial development. No cost information is available.

Beyond Rule-Based Decision Support: Power of Electronic Medical Records and Machine Learning-Based Algorithms

Typically, clinical decision support systems use limited data from patient history to generate severity scores and early warning signs using rule-based algorithms (194–197). Recent advances in machine learning further enable improvements to the timeliness and predictive accuracy of these computerized clinical decision rules. The application of powerful algorithms to large clinical data sets enables these technologies to learn site-specific contexts and novel relationships. As it increasingly becomes clear that a single biomarker may not have both the sensitivity and specificity to inform treatment (198), several groups have proposed algorithms that combine optimal subsets of biomarkers with rich electronic medical record (EMR) data (199–201). For example, a novel targeted real-time early warning score (TREWScore) predicts the development of septic shock in adult ICU patients 28 h before clinical onset (202), providing a superior alternative to the modified early warning score (MEWS) (203). This algorithm uses supervised machine learning to incorporate continuous sampling of a variety of physiological inputs, including platelets, ratio of blood urea nitrogen (BUN) to creatinine, arterial pH, temperature, bicarbonate, respiratory rate (RR), white blood cell count, systolic blood pressure (SBP), heart rate, and heart rate/SBP ratio (shock index). The TREWScore was developed by training the model with data from 13,000 patients to achieve an AUC of 0.83 with a sensitivity of 0.84 and a specificity of 0.67 on a validation set with 3,000 patients. Another recent study used machine learning to identify five optimal biomarkers for use with EMR data and reported an AUC of 0.81 with 10-fold cross-validation on 444 hospitalized adults. This study also attempted to stratify the sepsis syndrome to advance the fundamental understanding of the progression of this disease (199). Several other groups have demonstrated the validity of such algorithms in retrospective studies (204–206). However, only a few machine learning-based algorithms have been implemented in prospective studies beyond the development phase using retrospective chart review. Given this, it is difficult to discuss EMR-based machine learning algorithms in the light of the characteristics of ideal sepsis diagnostic tests. Nonetheless, we think that it is worth summarizing two of these promising approaches, which are under commercial development.

HeRO score. Heart rate characteristics (HRCs) have been used in clinics to provide an "early warning" of patient distress. Available commercially as the HeRO score algorithm (Medical Predictive Science Corporation, Charlottesville, VA), this technology uses signal processing and machine learning to identify subtle irregularities in heart rate variability. The HRC index used by HeRO was shown to reduce the mortality rate from 10% to 8% in an industry-sponsored randomized controlled clinical trial of 3,003 VLBW infants (207). However, the mechanisms for mortality reduction remain unclear. An independent, academic study of HRC monitoring in VLBW infants reported a greater utilization of antibiotics and more sepsis evaluations in a cohort with HRC monitoring than in controls without monitoring. This study also determined that there were no differences in the rates of blood culture-positive sepsis or clinically suspected sepsis as a function of the HeRO index (208). An additional single-center retrospective study reported that elevated HRC scores had a limited ability to detect bloodstream infection among neonates in the NICU, emphasizing that HRCs alone may not be adequate (209). Thus,

HeRO may represent another technology that could provide synergy in an integrated format with other diagnostic measures. The incremental costs of HRC monitoring are estimated to be US$2,000 per bed per year, or US$333 per patient, with an average length of stay for VLBW neonates (K. L. Karvonen, J. Vergales, R. A. Sinkin, and R. J. Swanson, presented at the Pediatric Academic Societies [PAS] Meeting, San Francisco, CA, 6 to 9 May 2017).

InSight. InSight (Dascena, Hayward, CA) uses routinely available clinical data to predict likelihood scores for user-specified outcomes. This algorithm has been validated for the early prediction of sepsis, severe sepsis, and septic shock in pediatric and adult populations across multiple centers (210–213). Conveniently, this algorithm requires only electronic health record data routinely available in clinics, with no need for continuous waveform data and extensive laboratory tests. This algorithm allows adaptation to site-specific data by training the model with baseline data from the proposed implementation center. It also allows modifications of gold-standard measures to predict patient conditions, such as in-hospital mortality (212) or patient stability (214). A recent trial across two surgical ICUs with 142 patients (75 controls) reported a 2.7-day reduction in the length of stay ($P = 0.04$) and a 12% reduction in in-hospital mortality ($P = 0.02$). Another study, reporting a 4-month experience in a 242-bed acute-care hospital, demonstrated a reduction in the length of stay of 0.43 days, along with a reduction in the mortality rate by 60.24% and a reduction in the rate of sepsis-related 30-day readmission, by 50%, postimplementation (215). Across these two studies, the algorithm score had a sensitivity and a specificity of 0.83 and 0.96, respectively, for sepsis. For severe sepsis, a sensitivity of 0.85 to 0.9 and a specificity of 0.9 were observed for adults. This algorithm has also been validated in pediatric patients ($n = 11,000$), reporting an AUC of 0.72 for predicting sepsis 4 h before onset and an AUC of 0.92 for predicting sepsis at onset (213). These results show promise for the early detection of sepsis in adult and pediatric patients. However, it remains to be seen if such methods will be equally successful for the neonatal population, where the physiology and pathogenesis of sepsis may be slightly different. For an individual ICU with 50 beds, InSight is predicted to annually save 75 additional lives and reduce sepsis-related costs by US$560,000 (216).

Machine learning techniques allow for the use of heterogeneous data sets to inform clinical decisions. The future should see the incorporation of EMR data with broad-based molecular detection technologies and clinical context to provide a significant increase in the reliability of these technologies. In this era of large-scale data integration, combining broad-based molecular techniques with EMR data represents tremendous opportunities for the timely and accurate diagnosis and management of sepsis as well as gains in insight into human disease mechanisms.

CONCLUSION

An exciting new era of molecular diagnostics for bloodstream infections is emerging through innovations in sample preparation, single-molecule detection methods, sequencing, and applications of machine learning. However, each emerging technology harbors unique benefits and drawbacks. For example, U-dHRM addresses the challenge of detecting pathogens in a low blood volume with high sensitivity while resolving polymicrobial infections, all in a potentially portable format and clinically actionable time frame. However, sample preparation and handling are still required, which increases the time to detection and may lead to some loss of sensitivity. Likewise, SeptiCyte provides a robust way to detect whether a pathogen is present based on the host response and provides this information in a time frame similar to that of U-dHRM but requires a higher volume of blood and initial sample preparation. The IC 3D technology is limited in the number of targets that it can detect in a single sample but is capable of skipping sample preparation entirely to accomplish the simplest and most direct testing from blood samples. This may be of significant value for rapidly tracking the spread of individual organisms in the context of outbreaks and hospital-acquired infections. Furthermore, in the era of big data, advances in the field of machine learning

can add patient-specific contextual information to each diagnostic test to potentially increase its sensitivity. The integration of host- and pathogen-targeted diagnostic technologies and their combination with EMR data sets using machine learning constitute a promising new frontier. Combining diagnostic technologies that build on distinct approaches could be a rapid way to improve positive and negative predictive power and truly impact antibiotic usage in the clinic. Together, these emerging technologies have the potential to identify microorganisms and provide relevant subspecies and antibiotic resistance information in a clinically relevant time frame that is much shorter than that currently required for blood culture. Such an integrated approach may overcome the limitations of each technology individually to facilitate targeted and precise antibiotic use.

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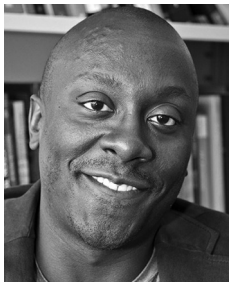

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