



Sepsis: Changing the Testing Paradigm

Mark D. Hughes, Vice President Strategy and Technology

Troy Galloway, President

Enterprise Analysis Corporation

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Introduction

There is a compelling need for more accurate and rapid information to improve the diagnosis and treatment of sepsis. Over the last decade at least 30 IVD companies have introduced, or are in the process of developing, diagnostic products to reduce the time to diagnose sepsis, identify pathogens and profile antibiotic susceptibility. This article provides a review of the current state of sepsis testing and the companies that are active in this arena.

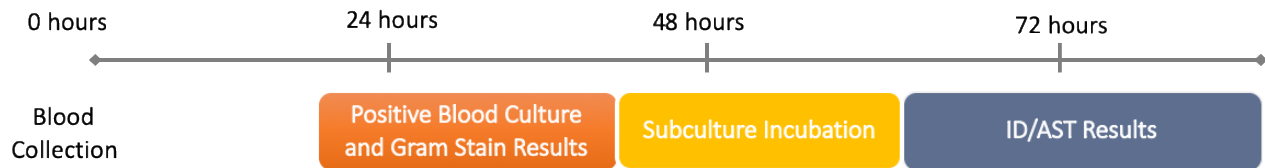
Background

Sepsis is an overwhelming and life-threatening immune response to infection. While sepsis is typically associated with bloodstream infections, it can occur with infections that have not entered the blood. In the US, there are an estimated 1.7 million cases of sepsis each year, resulting in more than 270,000 deaths.ⁱ According to the Agency for Healthcare Research and Quality, the healthcare costs incurred in the US from sepsis in 2013 reached nearly \$24 billion, roughly 6% of the nation's hospital bill, while these sepsis patients represented only 3.6% of all hospital stays.ⁱⁱ A new study published in the March 2020 issue of *Critical Care Medicine* estimates that sepsis-related costs for Medicare beneficiaries in the hospital and in skilled nursing facilities were at \$41.5 billion in 2018, up from \$27.7 billion in 2012.ⁱⁱⁱ Additionally, the Global Sepsis Alliance cites recent data claiming that in 2017 there were 49 million sepsis cases globally, resulting in 11 million deaths.^{iv}

With the knowledge that the mortality rate for sepsis increases by 7.6% with each hour of treatment delay,^v and in the absence of a reliable, rapid diagnostic test for sepsis, clinicians will treat suspected sepsis cases empirically with high dose, broad spectrum antibiotics. The decision to treat is based on clinical presentation and physiological parameters (e.g., temperature, heart rate, mental state and respiration), along with non-specific laboratory tests such as complete blood count, C-reactive protein (CRP), blood lactate and procalcitonin.

The blood culture is the current “gold standard” for bacteremia associated with a sepsis diagnosis. It is an imperfect standard as it is well known that blood cultures are frequently negative even though other tests and clinical signs point to sepsis. This can occur when there are too few bacteria in the sample, or the organisms are fastidious bacteria or fungi that are difficult to grow, or there may be a non-bacterial cause such as a virus. Further complicating the issue is the fact that blood cultures typically take 16-24 hours, and sometimes several days, before turning positive. Nonetheless, it remains the mainstay of diagnosis.

When a blood culture bottle turns positive (i.e., bacterial or fungal growth is detected), a Gram stain is performed and a preliminary report (Gram positive or negative along with organism morphology) is provided to the clinician. After subculture to plated media, organism identification (ID) and antimicrobial susceptibility testing (AST) are performed from the isolated colonies. An additional 36 – 72 hours is generally needed to report the complete ID and AST results. The graphic below illustrates the timeline for availability of results using conventional methodologies:



EAC notes that many microbiology laboratories have already adopted molecular technologies (e.g., BioFire FilmArray or Luminex Verigene) or MALDI-TOF (a form of mass spectrometry) that enable organism identification within 1-2 hours of obtaining a positive blood culture.

The Unmet Need

Given the time required to provide blood culture results, there is a clear need for fast and accurate laboratory testing for bacteremia and sepsis. The need is even more important in an age where antibiotic drug resistance is much more common and the arsenal of effective antibiotics available to treat resistant infections is more limited. Empiric, broad-spectrum antibiotic therapy administered to treat sepsis can contribute to the rise in antibiotic resistant strains. As a result, hospitals are implementing and following antimicrobial stewardship programs to optimize the use of antibiotics, protect patients from harm caused by unnecessary antibiotic use, and combat antibiotic resistance. There are both strong clinical and health economic cases to be made for rapid sepsis diagnosis, as it can translate into better treatment decisions leading to improved patient outcomes and reduced hospitalization costs.

The approaches to the ideal “*holy grail*” test (or panel) for the diagnosis of sepsis and/or bloodstream infection could include the following:

1. A rapid (one hour or less) “yes/no” sepsis test, such as an immune host-response assay, to reliably rule in or rule out sepsis.
 - Ruling out sepsis with a high negative predictive value (NPV) would prevent clinicians from starting unwarranted and expensive antibiotic therapy.
2. A rapid (within 3 hours of suspicion) organism ID or ID classification test to guide more tailored therapy.
 - This is a significant challenge because the breadth of causative organisms is enormous; 25 to 30 organisms account for the majority of sepsis cases, but that still leaves a very long list of organisms that occasionally result in sepsis.

3. A rapid (ideally within 1 hour of suspicion; 6 hours after the first dose of antibiotics may be acceptable) antibiotic susceptibility profile that would allow physicians to choose the most appropriate antibiotic therapy for the pathogen.

EAC has heard from many infectious disease physicians that the antibiotic susceptibility profile would be more powerful and definitive than the organism ID, but that the ID is still very useful in the absence of a significantly faster AST.

In addition to the need for a rapid pathogen ID/AST or “rule in/rule out” of sepsis in a patient presenting with symptoms, there are two other notable unmet needs:

- First, among patients already diagnosed and starting (or continuing) treatment, it would be very helpful to have a *prognostic* biomarker assay that could risk stratify patients and identify those that are progressing to septic shock. This would allow faster implementation of therapies such as vasopressor medications to avoid septic shock.
- Second, a test to identify patients in the early stages of sepsis or bacteremia *before* classic symptoms appear and *before* the clinician has a high index of suspicion of sepsis. Such a test would provide the ability to identify at risk patients at the earliest possible time when there is the greatest opportunity for improved outcomes and avoiding of ICU admissions.

In the absence of a definitive prognostic or predictive biomarker, a potentially valuable approach may be the use of machine learning algorithms that can analyze data such as vital signs, laboratory values, and comorbidities to help clinicians predict the risk of sepsis at early stages.

We now would like to share a summary of the latest developments in sepsis and bacteremia detection.

Sepsis and Bacteremia Detection: The Latest Developments and Diagnostic Products

A variety of new laboratory tests have been brought to market, or are still in development, to help provide more rapid information. We grouped these developments into the following categories:

1. Tests for faster organism ID and/or AST from a positive blood culture (reducing the time to result by eliminating the need for a subculture)
2. Tests to detect and provide an organism ID and/or AST from a blood sample (reducing the time to result by providing information before the blood culture test results)
3. Tests to rule in or rule out sepsis by detection of specific blood biomarkers, host-immune system response, or white blood cell morphology characteristics

Each of these categories is discussed in more detail below. For an overview of all of the companies and tests, refer to the table at the end of the article.

1) Rapid Identification and/or AST from Positive Blood Culture

The first category of tests are those that enable rapid identification of organisms directly from the positive blood culture, rather than waiting for the subculture. Several IVD manufacturers have introduced rapid “sample-to-answer” molecular tests that allow the microbiologist to identify the organism(s) in the positive blood culture in 1-2 hours. These molecular tests also detect the most common antibiotic resistant genes. While these tests are not the ideal “direct from whole blood” sepsis test, they do represent a step in the direction of faster, more accurate diagnosis.

Furthermore, several companies are working toward, or already have, a broad phenotype AST that can be performed on positive blood cultures, thereby reducing time to result by eliminating the overnight subculture step.

Accelerate Diagnostics

Accelerate Diagnostics (Tucson, AZ) has introduced the Accelerate Pheno system along with Accelerate PhenoTest BC kit which identifies pathogens from positive blood cultures in 1.5 hours using a molecular method called fluorescent in-situ hybridization (FISH). The Pheno system also performs AST on the identified organism using time lapse imaging of bacteria and computer algorithms with results in approximately 7 hours. The company received FDA granting of its *de novo* request in February 2017 and achieved revenues of more than \$9 million in 2019, representing more than 60% growth over the prior year. Accelerate now has installed several hundred of the Pheno systems which indicates growing acceptance of the product in the market.

Bruker Dalton and bioMérieux (MALDI-TOF)

A form of mass spectrometry called MALDI-TOF can be used for rapid identification of bacteria and fungi from positive blood cultures. There are two companies that supply MALDI-TOF equipment to microbiology laboratories: Bruker Daltonics (Bremen, Germany) with its Biotyper instrument and bioMérieux (Marcy-l’Etoile, France) with its Vitek MS system.

This technology is increasingly being adopted by microbiology laboratories for the identification of bacteria from isolated colonies as it is faster than traditional biochemical identification methods. Bruker recently obtained the CE-Mark for its Sepsityper kit to enable MALDI-TOF to be used directly from positive blood cultures. The company claims that the assay can identify 425 different bacteria and fungi in 15-20 minutes from a positive blood culture bottle. The company has also submitted for FDA clearance. Besides the obvious advantage of rapid results, MALDI-TOF also has a very low cost per test (once the instrument has been paid for). Additionally, some laboratories are performing MALDI-TOF directly from positive blood culture media in the form of a laboratory developed test (LDT) which they have developed and validated for internal use.

EAC estimates that several thousand MALDI-TOF instruments have been placed in microbiology laboratories globally over the last 10-15 years and that this adoption trend will continue.

BioFire

BioFire (Salt Lake City, UT), acquired by bioMérieux in 2014, received FDA clearance in 2013 for a blood culture ID test for its FilmArray system. The FilmArray BCID test is a “sample-to-answer” multiplex real-time PCR based test that detects 24 pathogens (including yeast) and 3 antibiotic resistance markers from a positive blood culture bottle. Results are available in approximately 1 hour. The company is developing a second generation BCID panel that will detect 43 bacteria, yeast and resistance genes. EAC surveys indicate that laboratories generally pay between \$120 and \$150 per test for the BCID assay.

Luminex

Luminex (Austin, TX) offers the Verigene system which provides a blood culture ID assay with results in 1.5-2 hours. The Verigene test has the flexibility to test for either Gram negative or Gram positive bacteria, using separate test cartridges. Both panels include selected resistance genes that are relevant to bloodstream infections. Luminex is currently introducing its new Verigene II system, which has random access capability and is more flexible and user-friendly. Although the blood culture panels are not yet available on the new Verigene II, EAC expects that these will eventually be added to the instrument menu along with the gastrointestinal and respiratory pathogen panels.

GenMark Diagnostics

GenMark (Carlsbad, CA) is a provider of automated, multiplex molecular diagnostic testing systems. Their ePlex platform is a sample-to-answer system for the diagnosis and disease management of bloodstream infections (BSI) that can lead to sepsis. In December 2018, the company announced FDA 510(k) market clearance had been granted for their ePlex Blood Culture Gram-Positive (BCID-GP) and Fungal Pathogen (BCID-FP) Panels, followed by the 510(k) clearance of the Blood Culture Identification Gram-Negative (BCID-GN) Panel in April 2019. The Gram negative panel detects 21 different bacterial targets and 8 antibiotic resistance genes while the Gram positive panel detects 20 targets and 4 antibiotic resistance genes. Processing time is approximately 1.5 hours for each panel.

iCubate

Yet another company with a molecular blood culture identification test is iCubate (Huntsville, AL). The company obtained FDA clearance for a multiplex Gram positive ID test in 2017 and followed it up with FDA clearance for a Gram negative ID test in July, 2019. These tests also detect the key resistance genes in Gram negative and Gram positive bacteria. The assays are run on the IC-System, an integrated multiplex PCR system that combines the sample preparation, amplification and detection using disposable test cassettes. A second module serves as the reader to provide the actual result of the test. The instrument has the ability to run 4 test cassettes in parallel. Test results are available in about 4 hours. The company also has a Mycobacteria test available for research use only.

Abacus Diagnostica

Another start-up company, Finland based Abacus Diagnostica Oy, is commercializing its GenomEra CDX PCR platform, a small desktop sample-to-answer PCR instrument that utilizes low cost disposable plastic test chips and time-resolved fluorescence for detection. Up to 4 tests can be run simultaneously in the instrument with test results in about 1 hour. The test menu includes 2 CE Mark assays for pathogen identification of positive blood cultures: a combination test for both methicillin- sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) and a separate assay for *Streptococcus pneumoniae*.

Cepheid

Cepheid (a Danaher company) has a 1 hour sample-to-answer test on its GeneXpert system for detection of *Staphylococcus aureus*. The test, called Xpert MRSA/SA, is specifically designed to detect MSSA and MRSA direct from Gram positive blood cultures. This test has been on the market for many years and is useful when there is high suspicion of a Staph infection from the Gram stain.

Q-linea

Q-linea AB (Uppsala, Sweden) is a development stage company founded in 2008. The original intention of the company was to develop a rapid pathogen ID and AST system directly from the patient's blood sample. The company had gone as far as developing a prototype instrument for the ID whereby it could provide a test result in 4 hours directly from blood. However, the company changed its strategy in 2017-18 to focus on the AST portion of the business opportunity.

The company has developed a prototype instrument platform called ASTar that performs a phenotypic AST covering 48 antibiotics within 3-6 hours from a positive blood culture. The ASTar is fully automated, requires only 1 minute of hands on time and delivers a full Minimum Inhibitory Concentration (MIC) result similar to traditional AST/MIC microbiology systems. The system is random access and can run up to 12 samples at the same time. It can be used with other types of specimens as well as colony isolates. In February 2020, Q-linea announced a global partnership with Thermo Fisher Scientific for commercialization and distribution of the ASTar platform.

Astrego Diagnostics

Astrego is a start-up diagnostics company based in Uppsala, Sweden. Similar to Q-linea, it is focused on the development of a rapid system for AST testing. It has developed a prototype product called Captiver, a fully automated, small desktop instrument that produces a full phenotypic AST result from a positive blood culture in 5-60 minutes. It can also be used for urinary tract infections (UTI) with AST results in under 30 minutes. This is far faster than conventional phenotype AST testing from an isolated bacterial colony.

The instrument platform uses small microfluidic chips with dozens of micro-channels that contain antibiotics (or none at all). The micro-channels are continuously monitored for growth of the bacteria and an algorithm is applied to determine the antibiotic susceptibility profile. The company has not announced a launch date for the product yet. Notably, Japan based Sysmex announced an investment in the company on January 2020.

Specific Diagnostics

Specific Diagnostics (Mountain View, CA) is a development stage company that is working to develop a 4.5 hour phenotypic AST test from positive blood culture bottles or bacterial colony isolate dilutions. The company has developed a patented biosensor array platform called Reveal. This is described as a small molecule sensor (SMS) array that can detect volatile organic compounds (VOCs) released by bacteria at concentrations as low as 10 parts per billion. The presence of these VOCs results in a color change on the sensor that is detected in a scanning instrument. The type and quantity of VOCs relates back to the antibiotic susceptibility profile. The company claims that the Reveal platform requires only 2 minutes of sample preparation and that it can be configured into a low or high throughput modular system at a low cost.

SeLux Diagnostics

SeLux Diagnostics (Boston, MA) is a development stage company founded in 2014. SeLux is a rapid AST technology that they refer to as Next Generation Phenotyping or NGP. The company claims that the new NGP platform will provide “high throughput and fully automated AST results” for up to 50 antibiotics. SeLux also claims the NGP platform is 2-4 times faster than current state of the art AST technology and will provide “same shift” results for AST. The company is also working on a second generation platform that will provide same day results for sepsis and UTI direct from sample.

The company has raised \$25 million in Series B financing and was recently awarded a contract valued at up to \$45 million from the Biomedical Advanced Research and Development Authority (BARDA).

2) Results Directly From a Blood Sample

The next category of tests are those that provide results directly from a whole blood sample, thereby eliminating the need to wait for the blood culture to become positive. Companies in this category are using newer technologies including PCR, next generation sequencing (NGS), and other variations of molecular technologies to perform a rapid, direct organism identification. There are a few companies (e.g., T2 Biosystems and Seegene) that include resistance marker information in addition to the organism identification. There are also a few companies with technologies in development (e.g., Roche GeneWeave, Momentum Bioscience) to provide a full AST.

EAC's field checks with laboratories indicate that, while there is great interest, the tests are not yet widely adopted as labs need to gain more confidence in test performance and are able to justify the cost and labor versus impact to patient outcomes.

Roche Molecular

The Roche Molecular SeptiFast PCR test kit was first introduced in 2006 with CE-IVD certification outside the US. The test is performed on the LightCycler 2.0 instrument and can detect 25 different bacteria and fungi that account for over 95% of bloodstream infections.

The SeptiFast test is designed to be used in conjunction with standard microbiology procedures and clinical presentation. Test results take 4-6 hours including the time for nucleic acid extraction. The LightCycler is a batch instrument and is, therefore, not ideal for running one sample at a time on demand which reduces the positive impact this test could have.

More recently, in 2015, Roche Molecular Systems acquired US-based GeneWeave for \$190 million with additional payments of up to \$425 million total if GeneWeave meets all its development milestones.

GeneWeave has been developing a fully automated platform using a proprietary "Smarticle" technology for AST testing. Simply put, these are DNA delivered bio-particles that enter bacterial cells and cause live bacteria to produce luciferase, thereby allowing phenotypic detection to an antibiotic in 3-4 hours. A bioluminescent signal is detected by the instrument. The initial commercial focus seems to be AST but the technology appears capable of performing pathogen identification as well.

Molzym GmbH

Molzym GmbH (Bremen, Germany) introduced a CE Mark direct-from-blood sepsis test called SepsiTst-UMD in 2008. It can detect 345 different genus and species of bacteria and fungi from 1ml of whole blood or other types of samples (e.g., spinal fluid). It is intended for use with other standard diagnostic tests. After the DNA purification, the assay requires PCR steps to amplify the 16s and 18s rRNA genes and this is followed by Sanger sequencing analysis for the pathogen identification. The company states on its website that its assay has detected more than 1,350 different species of bacteria and fungi from a variety of specimen types.

Using its patented DNA extraction method, the assay procedure removes the host (patient) DNA from the blood (or other sample) while the microbial DNA from live bacteria or fungi is left behind and can be concentrated. As a result, the company claims that it is able to frequently detect microbial infections in culture negative patients.

While interesting, the assay configuration is very labor intensive and basically requires many hours to run and, therefore, it is not very practical for STAT testing in most routine microbiology laboratories.

Momentum Bioscience

Momentum Bioscience (Cardiff & Oxford, UK), founded in 2008, is a molecular diagnostics company focused on developing a rapid test to aid in the diagnosis of sepsis. The company's current test in development, SepsisSTAT, reports the absence or presence of viable bacteria and yeast direct-from-blood in under 3-hours and provides a clinically relevant organism identification within the same period.

Momentum has developed a highly efficient capture and purification process demonstrating microorganism extraction from blood at as little as 1 CFU/mL and which, combined with their modified qPCR technology called Enzymatic Template Generation and Amplification (ETGA), amplifies only viable microbial cells. The company believes that their core purification and ETGA technologies have additional benefit to down-stream applications such as rapid AST and NGS. Momentum has not yet announced when they expect the test to be available.

Mobidiag

Mobidiag (Espoo, Finland) introduced the first blood culture pathogen ID test in 2008 with its Prove-it Sepsis test. The product procedure required a separate DNA extraction step followed by standard PCR amplification and then detection on a DNA array that detected 84 bacteria, fungi and antibiotic resistance markers in 3 hours. This was a "first generation" sepsis test and the company subsequently halted sales of this product to focus on developing more automated solutions.

In May 2018, the company announced new investor funding of \$1.74 million from Business Finland for development of a next generation rapid whole blood sepsis test on its Novodiag sample-to-answer molecular instrument platform aimed at producing results within 2 hours. The test will widely cover the detection of pathogens causing sepsis and primary antimicrobial resistance markers. In January 2020, Mobidiag announced that it received an additional \$1.68 million in outside funding to support development of its sepsis assay.

Seegene

Seegene (Seoul, South Korea) has a direct from whole blood multiplex PCR pathogen identification test on the market outside the US. The test, called Magicplex, detects 90 different bacterial and fungal species including 3 drug resistance markers. Test results are available in approximately 3 hours (excluding sample preparation and DNA extraction time). The assay can be run on several "open" real-time PCR instruments such as the Bio-Rad CFX96 and the Thermo AB7500. The company is currently working with various partner companies to deliver a more automated assay format for many of its assays.

T2 Biosystems

T2 Biosystems (Lexington, MA) has developed a detection method that uses proprietary magnetic resonance technology along with superparamagnetic particles that are coated with pathogen specific binding agents. According to the company's website, this technology can identify molecular targets within patient samples without the need for purification or extraction of target molecules from the sample.

T2 Biosystems has launched two direct-from-blood organism identification test panels to date. The first panel, T2Candida, received FDA clearance in 2014 and detects Candida species from whole blood with results in 3-5 hours. The company claims that the test is 91% sensitive and can detect as few as 1 CFU/ml of Candida in a blood sample. The second panel, T2Bacteria, received CE marking in 2017 and FDA clearance in 2018. T2Bacteria detects six of the most serious sepsis causing Gram negative and Gram positive bacterial species with results in 3-5 hours.

As of the writing of this article, T2Candida and T2Bacteria are the only FDA-cleared and CE-marked panels for detection of sepsis-causing bloodstream infections directly from a patient's blood sample. In November 2019, T2 Biosystems received CE marking for its T2Resistance Panel, another direct from whole blood test which detects 13 of the most commonly founded antibiotic resistant genes associated with bloodstream infections.

The company had product revenues of approximately \$5.3 million in 2019 and continues to seed the market with their new technology.

Qvella

Qvella (Ontario, Canada), was founded in 2009 with a vision of dramatically reducing time to result in microbiology. The company has developed a proprietary array technology called Field Activated Sample Technology (FAST) with the goal of providing clinicians with real-time test results. The company plans to launch its first product, FAST-Prep PBC, in the spring of 2020. The product is designed to prepare a Liquid Colony to be equivalent to a colony grown on a plate for downstream processing from a positive blood culture in under 20 minutes.

Also in development using the core FAST technology is FAST-ID, a rapid, multiplex sample-to-answer PCR system that is intended to deliver a pathogen ID result in about 1 hour from a blood draw. FAST-ID features closed tube blood sampling, disposable test cartridges, two minutes of hands on time and automated concentration and lysis of bacterial and fungal pathogens. The company says that their platform utilizes a "novel electrical lysing and sample preparation" method. The company previewed the FAST-ID system at the Association for Molecular Pathology (AMP) meeting in November, 2019.

Qvella also announced a partnership with the Biomedical Advanced Research and Development Authority (BARDA) of the US Department of Health and Human Services (HHS). The BARDA DRIVE Solving Sepsis partnership with Qvella is an initiative to develop a 1 hour host immune response assay to run on the FAST-ID platform. The company raised \$20 million in Series B financing in the fall of 2017. Product development continues but a product launch date has not been announced. The company is expected to enter FAST-ID into clinical evaluation in 2020.

DNAe

London based DNAe has developed a system for bloodstream infections called LiDia. The company has presented poster sessions at recent microbiology and molecular meetings claiming that it can detect pathogens in blood in concentrations as low as 1 CFU/ml. The LiDia is a closed system that performs the DNA extraction and a nested PCR pre-amplification in a self-contained test cartridge. According to their website, any DNA or RNA from a pathogen can be detected using standard semiconductor technology. Simply put, hydrogen ions that are released during DNA/RNA sequencing or amplification are detected by electrical signal using a complementary metal oxide semi-conductor (CMOS) chip. The company claims that test results, including AST, can be determined in 3 hours.

The company has a contract with BARDA that is worth up to \$52 million, assuming it meets all of its progress milestones.

Karius

Karius (Redwood City, CA) is a new entrant in the sepsis testing market. The company operates a licensed CLIA certified laboratory and uses NGS to detect cell free microbial DNA (cfDNA) from a 5 ml plasma sample.

The company claims to detect more than 1,000 different bacteria, viruses, fungi and parasites from a blood sample with results available in 1 day (from blood sample receipt at the lab). The test detects microbial cfDNA from organisms in the bloodstream or even deep-seated infection in organs. The Karius test also provides a quantitative indication of the abundance of organisms in the sample and therefore may be a measure of the severity of infection.

In a study published in 2017 in the journal *Open Forum Infectious Diseases*, the authors (who are company employees) claim that the NGS test detected pathogens in septic patients three times more often than blood culture. In another study described on the Karius website, 350 suspected sepsis patients were tested by NGS and traditional blood culture. The company claimed to have 93% sensitivity versus blood culture. The list price for the test is \$2,000.

This test may prove especially valuable in patients whose blood contains pathogens that are difficult to grow in culture (e.g., *Bartonella* species) and for patients with Fever of Unknown Origin.

In February, 2020, Karius announced that it had raised an additional \$165 million in a Series B financing round.

PathoQuest

France based PathoQuest, founded in 2010 as a spinoff of the Pasteur Institute, has developed a direct-from-blood intact organism detection NGS based solution, iDTECT.

iDTECT Blood combines proprietary sample preparation protocols, QC reagents and bioinformatic analyses to generate over 1,600 bacterial species and virus identifications from metagenomically sequenced blood samples. Both RNA and DNA viruses are detected. Detection of antimicrobial resistance genes will be added in the next version of the product. A first version of the test has obtained the CE Mark in Europe, and is available through a collaboration with selected hospitals.

An automated version of iDTECT dramatically reduces sample preparation manipulation and hands-on time with improved read efficiency and reduced turnaround time (1-2 days). For the US market, PathoQuest plans to introduce its solution as a laboratory developed test (LDT) through partner laboratories prior to FDA validation. In 2019, the company announced it has engaged in US-based iDTECT evaluations in immunocompromised patients with febrile neutropenia, in collaboration with key hospitals including Mayo Clinic and Memorial Sloan Kettering Cancer Center.

In 2019, the company raised €8 million from venture partners and is planning to develop iDTECT assays from different biological samples.

Noscendo GmbH

Noscendo GmbH (Duisburg, Germany) is similar to Karius and PathoQuest in that it uses NGS to identify pathogens directly from a blood sample. Noscendo has developed the DISQVER platform which utilizes NGS technology and a proprietary bioinformatics program to identify cell free DNA (cfDNA) in plasma samples. DISQVER is a CE certified medical device due to the proprietary bioinformatics. The DISQVER platform has the ability to distinguish human host DNA or RNA from bacterial or viral DNA or RNA as well as the ability to distinguish between contaminating or commensal organisms and actual pathogens by using statistical analysis of the sequences. The company states that they are able to detect nucleic acid in plasma samples for more than 1,500 bacteria, viruses, fungi and parasites.

The company is pursuing two business models. In the first model, the customer/physician sends an 8.5mL blood sample via courier to the Noscendo laboratory where all of the testing and analysis is performed and a report is returned indicating which organisms were found and their relative significance. The time frame for receiving the report is 36-48 hours after sample collection. The second model is an “enabling” model whereby individual hospitals or private laboratories that have NGS equipment can perform the test themselves after receiving training and the bioinformatics software from Noscendo.

In January, 2020, the company announced a partnership with the University Hospital Bonn to expand the DISQVER platform to include antibiotic resistances genes.

3) Host Response and Biomarker Detection

The third category of tests are those that detect a host immune response for sepsis or measure the presence of a blood-based biomarker.

While several markers have been available and in use for many years, they lack the required sensitivity or specificity to drive widespread adoption for sepsis diagnosis. For example, procalcitonin (PCT) is available on many laboratory immunoassay analyzers and even some point-of-care platforms. However, PCT is not an ideal sepsis diagnostic and not all institutions have adopted it for routine incorporation in a sepsis work up. Further, it does not eliminate the need for blood culture and antimicrobial susceptibility testing or other indicator tests of sepsis. Therefore, the search has continued for more sensitive and specific markers.

Immunexpress

Immunexpress (Seattle, WA), has been working toward a novel direct-from-blood rapid sepsis host response technology for many years. In February 2017, Immunexpress received FDA 510(k) clearance for the SeptiCyte LAB test as an aid in differentiating patients with true sepsis from patients that have a systemic inflammation condition called systemic inflammatory response syndrome (SIRS).

The Immunexpress SeptiCyte test is an RNA-based qPCR test that looks at a patient's own immune response to infection by measuring the expression of four genes involved in the immune inflammatory response. In 2018, Immunexpress announced a collaboration with Belgium-based Biocartis to put the SeptiCyte host response sepsis test (SeptiCyte RAPID) on the Biocartis Idylla point-of-care platform. Idylla is a fully integrated sample-to-answer platform that can produce a SeptiCyte test result in 1 hour from less than 1 ml of blood. In March 2020, Immunexpress received CE Marking of SeptiCyte RAPID and announced a long-term commercialization partnership with Biocartis in Europe. Immunexpress expects FDA 510(k) clearance for the test in the 2nd half of 2020.

Beckman Coulter

Beckman Coulter (Brea, CA), a subsidiary of Danaher and a longtime market leader in hematology testing, announced in May 2018 that it had received CE Mark for its Early Sepsis Indicator test. This was followed by FDA clearance in April, 2019. This test examines morphological characteristics of monocytes as part of a standard complete blood count (CBC) with differential, which should make it easy and cost-effective for institutions to adopt. The change in the morphology of the monocytes is a potential indicator of sepsis.

While the test is not an absolute indicator, a positive result indicates a higher probability of sepsis while a negative result indicates a lower probability. According to the company's press release, the test serves as an important tool for emergency department doctors to detect early sepsis or the risk of developing sepsis when the clinician would otherwise not have a high suspicion.

Abionic

Abionic SA (Lausanne, Switzerland), founded in 2010, has developed a nanotechnology based point-of-care platform called the abioSCOPE. The abioSCOPE is a small, touchscreen, portable desktop instrument that uses a miniaturized fluorescence microscope for detection. The technology utilizes a nanofluidic biosensor where a fluorescent immunoassay is performed within the nanofluidic channel in minutes. A special test cartridge, called IVD Capsule, requires application of a 50 µl sample of whole blood and yields test results in 5 minutes. The company claims up to 14 analytes can be multiplexed from a single drop of blood. This technology is being applied to a broad range of analytes in blood such as allergy, cardiac markers, thyroid assays, D-dimer, and many more.

For sepsis, the company received CE Mark for a proprietary sepsis test called pancreatic stone protein (PSP) test, a host response biomarker released from the pancreas in the early stages of sepsis. The clinical studies to date indicate that the PSP test can help to identify sepsis patients at an early stage in the ICU and emergency department. In September 2019, Abionic announced the results of a study of 300 ICU patients showing that its PSP test correlated well with the development of sepsis with earlier detection than current methods. A US trial is now underway that will support a 510(k) FDA submission.

Cytovale

Cytovale (San Francisco, CA) is a start-up company developing a sepsis test on a microfluidic platform that examines structural changes to white blood cells when dysregulation of the immune system occurs. The first application for the technology is a rapid test for sepsis for use in the emergency department. In October 2019, Cytovale announced it had received an additional \$15 million in financing to support sepsis test development; this included \$7.4 million in a Series B round and \$7.6 million from a BARDA contract.

Inflammatix

Another company developing a host response immune assay is Burlingame, CA based start-up Inflammatix. The company, founded in 2016, is developing an 18 gene host response molecular assay called HostDx Sepsis that can presumably distinguish bacterial from viral infection in suspected sepsis patients as well as stratify patients based on severity and assess 30-day mortality. The assay uses isothermal molecular technology to look at gene expression and combines this with a machine learning algorithm, potentially in a point-of-care setting. This test is still in the development stage and we suspect that it will likely be two more years before it gains regulatory approval.

In January 2020, the company announced that it had obtained an additional \$32 million in Series C financing. The company also announced in late 2019 that it signed a contract with BARDA. The contract is worth up to \$72 million, the funds of which will be used to help develop its HostDx Sepsis test as well as its HostDx FeverFlu assay.

MBio Diagnostics

MBio Diagnostics (Boulder, CO) is in the early stages of commercializing its point-of-care immunoassay platform called LightDeck. This is a compact and portable instrument that incorporates planar waveguide technology with fluorescence detection. Tests are performed on the LightDeck one at a time using low cost disposable test cassettes. The company is pursuing a wide range of applications in human clinical medicine, veterinary medicine, food safety and environmental testing.

Regarding sepsis, the company announced in August 2019 that it has been awarded 2 separate contracts for development of an immunoassay based sepsis test. The first is a Phase II Small Business Innovative Research (SBIR) grant from the National Institutes of Health (NIH). MBio will work in collaboration with Cincinnati Children's Hospital Medical Center (CCHMC) to assess the feasibility of a 5 biomarker blood test panel for use in pediatric sepsis cases. The 5 biomarkers are heat shock protein 70, interleukin-8, matrix metalloproteinase-8, C-C chemokine ligand 3, and granzyme B.

The second contract is with the Henry M. Jackson (HMJ) Foundation for Advancement of Military Medicine. This research contract is a collaboration with the Austere Environment Consortium for Enhanced Sepsis Outcomes (ACESO), which is a project sponsored by the Naval Medical Research Center. The research will focus on development of a rapid, quantitative assay combining an algorithm with multiplex detection of 10 blood based biomarkers. The specific biomarkers or time to result were not specified, but it is intended to be a rapid, point-of-care test for military field use.

Mitsubishi Chemical

Mitsubishi Chemical of Japan has been selling a 15 minute, whole blood biomarker assay for presepsin that runs on its automated desktop chemiluminescent immunoassay analyzer called PATHFAST. Presepsin, also called soluble CD14 subtype, is a glycoprotein expressed on the membrane surface of monocytes, macrophages and granulocytes. The presepsin ends up being released into the bloodstream by secretion following phagocytosis by white blood cells. Clinically, it is similar to procalcitonin in that measuring presepsin levels can aid in the prognosis of sepsis and monitoring response of the patient on antibiotics. There is some evidence that indicates that it increases in the blood earlier than procalcitonin and has higher sensitivity and specificity. Mitsubishi has been selling the assay outside the United States since about 2015.

Mologic

UK-based Mologic is yet another company developing a rapid whole blood biomarker immunoassay panel for sepsis. The company has a great deal of expertise in the field of lateral flow immuno-chromatography and is working to develop a far more sensitive version of the technology. Among its numerous development projects is a multimarker whole blood assay for sepsis. The aim is to have a rapid 10 minute test with a high Negative Predictive Value (NPV) to rule out sepsis. The assay will measure 6 different biomarkers (which are not specified) using lateral flow immunochromatography and a small desktop reader. The company is currently collaborating on a sepsis trial with University College London Hospitals.

Nanōmix

Nanōmix, based in Emeryville, CA, is a development stage company that has developed a handheld electrochemical nanosensor based detection platform called eLab for point-of-care use. In December 2019, Nanōmix was granted the CE Mark for its sepsis assay, called the S1 Assay, which measure 3 biomarkers in whole blood: lactate, CRP and Procalcitonin. The assay time is 11 minutes, although the current version of the test is *not* whole blood. The company states that it is working on a whole blood version that will be ready in 2020.

The company plans to launch outside the US in early 2020 using distributors and plans to file an FDA submission in Q1, 2020.

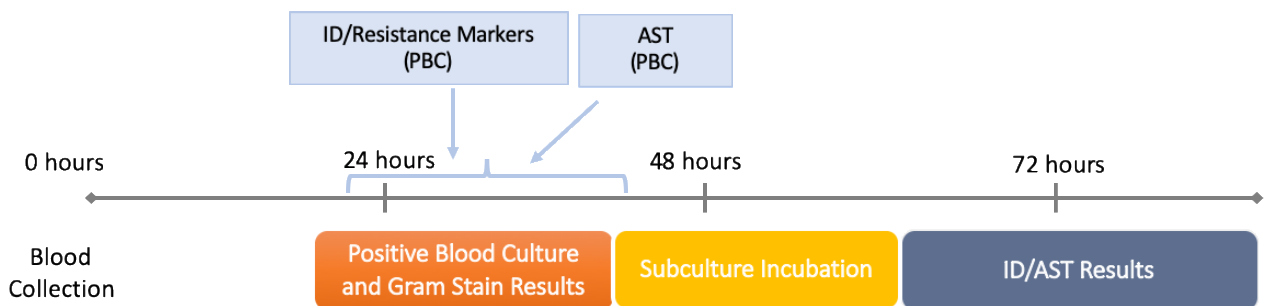
Sphingotec

Sphingotec GmbH (Henningsdorf, Germany) was founded in 2002 with the mission of discovering new biomarkers for disease. The company has developed a new immunoassay test, bio-ADM, which measures a bioactive adrenomedullin, a peptide hormone that is involved in regulating vasodilation and vascular leakage of blood vessels. As such, it can be used in the prediction and diagnosis of septic shock and to support clinical decisions to give vasopressor therapy. A rise in the blood level of bio-ADM usually occurs prior to the onset of septic shock.

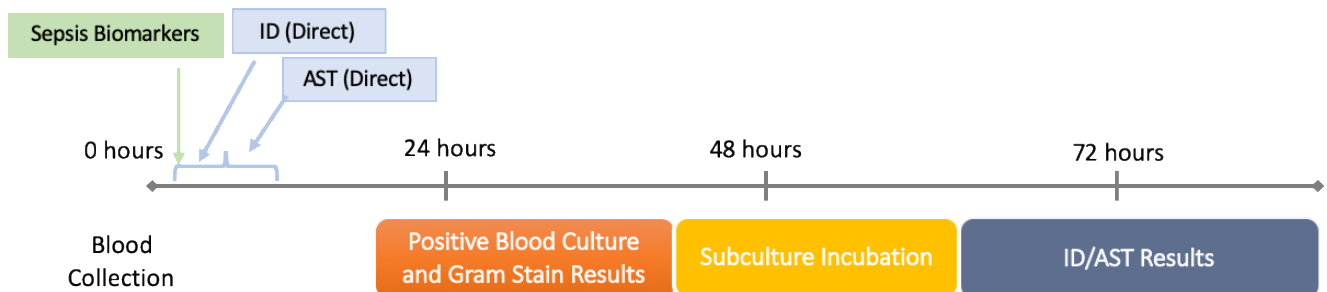
The company recently announced a collaboration with US based Nexus Dx (San Diego, CA) to develop its assays for use on the Nexus Dx IB10 microfluidic POC instrument. This instrument can produce immunoassay results at the point-of-care in as little as 20 minutes.

Summary and Conclusions

Significant progress has been made in sepsis testing in recent years, yielding faster ID and AST results and improved patient monitoring with biomarkers like procalcitonin. These advances have translated into clinicians being able to make better-quality, actionable treatment decisions with *potentially* improved outcomes for patients. EAC observes that the information paradigm has already begun to shift as more laboratories take advantage of these tests to obtain organism ID and susceptibility/resistance information more quickly than with traditional methods.



The most significant paradigm shift will occur of course when testing for sepsis biomarkers, organism identification and AST can be performed rapidly and reliably on whole blood specimens that are taken directly from the patient at the initial clinical presentation.



To date, the first generation of whole blood rapid sepsis tests have not been broadly adopted as they are not well suited to on demand stat testing due to their long turnaround times and labor requirements. However, many second generation whole blood sepsis assays are in development with the potential to lead to this paradigm shift in the way patients are diagnosed and treated.

Acceptance and adoption of new tests by clinicians, laboratorians and healthcare institutions will depend on the degree to which they meet clinical and operational criteria, including:

- Organism detection and ID test performance, including the rate of false positive or false negative results and the comparison of organism detection results to blood culture (which can be problematic given that blood culture is an imperfect gold standard)
- AST test performance and error rates compared to traditional AST methods from subculture
- Sensitivity and specificity of biomarkers for prediction, diagnosis, and/or prognosis
- The degree to which a test may be able to replace blood culture versus being an “add on” test
- Ease of acquisition and ease of use of testing platforms
- Ability to show clinical and economic outcomes such as faster patient recovery, reduced length of hospital stay, and improved antibiotic stewardship
- Test cost

EAC believes that in the fight to better predict, diagnose and treat sepsis, there will be room for multiple tests and approaches. This will most likely involve a combination of a rapid host response immune test, biomarkers (and potentially machine learning algorithms) for tracking prognosis, progression or remission, and rapid pathogen identification coupled with rapid antibiotic resistance testing.

A multi-billion dollar market opportunity is available to the companies that can contribute to the goal to deliver rapid, accurate sepsis prediction, diagnostics and treatment. With the array of technologies that are now available or are in development, the industry is making significant progress toward achieving this goal.

ⁱ Rhee C, et al. Incidence and trends of sepsis in US hospitals using clinical vs claims data, 2009-2014. *JAMA*. 2017;318(13):1241-1249. doi:10.1001/jama.2017.13836

ⁱⁱ Torio CM, Moore BJ. National inpatient hospital costs: the most expensive conditions by payer, 2013. Stat. Brief #204. HCUP. AHRQ. May 2016. <https://www.hcup-us.ahrq.gov/reports/statbriefs/sb204-Most-Expensive-Hospital-Conditions.pdf>

ⁱⁱⁱ Buchman TG, et al. Sepsis among Medicare beneficiaries: 1. The burdens of sepsis, 2012-2018. *Crit Care Med*. 2020;48(3):276-288. doi: 10.1097/CCM.0000000000004224.

^{iv} Global-Sepsis-Alliance. New study shows deadly sepsis rates are double prior estimates – *Global Burden of Sepsis* says poor countries and children hit hardest. January 16, 2020. <https://www.global-sepsis-alliance.org/news/2020/1/16/the-lancet-sepsis-associated-with-1-in-5-deaths-worldwide-double-prior-estimates-children-and-poor-regions-hit-hardest-global-burden-disease-study-kristina-rudd>

^v Kumar A, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med*. 2006;34(6):1589–1596. doi: 10.1097/01.CCM.0000217961.75225.E9

Summary of Companies and Technologies (Grey: tests performed from positive blood culture: Yellow: ID and/or AST tests from whole blood; Green: sepsis biomarkers)

| Company | Product or Platform | Status | Technology | Time to Result (approximate) | Specimen Type | Pathogen ID | Genetic Resistance Markers | Full AST | Host Response | Biomarker/ Other |
|------------------------|-----------------------------------|-------------|------------------------------|--------------------------------|------------------------------------|-------------|----------------------------|----------|---------------|------------------|
| BioFire | FilmArray | On Market | RT-PCR | 1 hour | Blood culture | Yes | Yes | No | No | No |
| Bruker | BioTyper | On Market | MALDI-TOF | 15-20 minutes | Blood culture | Yes | Yes | No | No | No |
| Luminex | Verigene | On Market | Gold nanoparticle microarray | 2 hours | Blood culture | Yes | Yes | No | No | No |
| GenMark | ePlex | On Market | PCR | 1.5 hours | Blood culture | Yes | Yes | No | No | No |
| Accelerate Diagnostics | PhenoTest | On Market | FISH | 1.5 hours (ID)/6-7 hours (AST) | Blood culture | Yes | No | Yes | No | No |
| Cepheid | Xpert MRSA/SA | On Market | RT-PCR | 1 hour | Blood culture | Yes | Yes (MRSA) | No | No | No |
| iCubate | iC-System | On Market | ARM-PCR | 3-4 hours | Blood culture | Yes | Yes | No | No | No |
| Abacus Diagnostica | GenomEra | Development | RT-PCR | 1 hour | Blood culture | Yes | Yes | No | No | No |
| Q-linea | ASTar | Development | Phenotype AST | 3-6 hours | Blood culture | No | No | Yes | No | No |
| Astrego Diagnostics | Captiver | Development | Phenotype AST | 5-60 minutes | Blood culture | No | No | Yes | No | No |
| Specific Diagnostics | Reveal | Development | Phenotype AST | 4.5 hours | Blood culture | No | No | Yes | No | No |
| SeLux Diagnostics | Next Gen Phenotyping | Development | Phenotype AST | ~4 hours | Bacterial isolates & direct sample | No | No | Yes | No | No |
| Mobidiag | Novodiag | Development | PCR | 1-2 hours | Whole Blood | Yes | NA | No | No | NO |
| Momentum Bioscience | NA | Development | ETGA | 3 hours | Whole blood | No | No | No | No | No |
| Roche Molecular | SeptiFast | On Market | RT-PCR | 4-6 hours | Whole blood | Yes | Yes | No | No | No |
| Roche GeneWeave | Smarticle | Development | DNA bioparticles | 3-4 hours | Whole blood | Yes | No | Yes | No | No |
| Seegene | Magicplex | On Market | RT-PCR | 3-4 hours | Whole blood | Yes | Yes | No | No | No |
| Molzym | SepsiTest | On Market | PCR | 4-5 hours | Whole blood | Yes | Yes | No | No | No |
| T2 Biosystems | T2Bacteria/T2Candida/T2Resistance | On Market | Magnetic Resonance | 3-5 hours | Whole blood | Yes | Yes | No | No | No |
| Immunexpress | SeptiCyte | On Market | Gene expression | 4-6 hours | Whole blood | No | No | No | Yes | No |
| Karius | Karius Test | On Market | NGS | >24 hours | Whole blood | Yes | No | No | No | No |
| PathoQuest | iDTECT | On Market | NGS | 48 hours | Whole blood | Yes | Yes | No | No | No |
| Noscendo GmbH | DISQVER | On Market | NGS | 36-48 hours | Whole blood | Yes | Yes | No | No | No |
| Qvella | FAST-ID BSI | Development | Multiplex PCR | <1 hour | Whole blood | Yes | Yes | No | No | No |
| DNAe | LiDia | Development | PCR and semi-conductor chip | 3 hours | Whole blood | Yes | Yes | Yes | No | No |
| Inflammatix | HostDx Sepsis | Development | Gene expression profile | <60 minutes | Whole blood | No | No | No | Yes | No |
| Cytovale | NA | Development | White Blood Cell morphology | <10 minutes | Whole blood | No | No | No | No | Yes |
| Abionic | abioSCOPE | Development | PSP Biomarker -Immunoassay | 5 minutes | Whole blood | No | No | No | No | Yes |
| Sphingotec | Nexus IB10 | Development | Bio-ADM- Immunoassay | 20 minutes | Whole blood | No | No | No | No | Yes |
| Beckman Coulter | DxH 900/DxH 690T | On Market | Monocyte morphology | <5 minutes | Whole blood | No | No | No | No | Yes |
| MBio Diagnostics | LightDeck | Development | Planar wave guide IA | 10 minutes | Whole blood | No | No | No | Yes | Yes |
| Nanōmix | eLab | Development | Electrochemical sensor | 11 minutes | Plasma/Serum | No | No | No | No | Yes |
| Mologic | NA | Development | Lateral Flow IA | 10 minutes | Whole blood | No | No | No | No | Yes |
| Mitsubishi | Pathfast | On Market | Chemiluminescent IA | ~15 minutes | Whole blood | No | No | No | No | Yes |



About Enterprise Analysis Corporation (EAC)

EAC helps diagnostic companies successfully commercialize new technologies. From investment decisions to product development requirements, and from go-to-market to market expansion strategies, we provide the information and insights needed for companies to make sound decisions.

EAC Facts:

- Deep diagnostic knowledge with more than 100 years of combined IVD experience
- Over 1,400 projects completed for more than 160 client companies
- Global network: US, Europe, Asia, and Latin America
- More than 30 years in operation

In addition to strategic consulting in areas such as market opportunity assessment and portfolio analysis, we conduct primary research using proven methodologies including conjoint analysis, outcome research, pricing and workflow studies, user satisfaction assessments, and others.

For more information, visit our website at www.eacorp.com or contact us at jkatz@eacorp.com or 203.348.7001

About the Authors

Mark Hughes, Vice President Strategy and Technology



Mark Hughes joined EAC in 1997 and has more than 35 years of experience in the clinical diagnostics industry. His projects have included technology assessment, market analysis, business strategy and due diligence for major diagnostic, pharmaceutical and start-up biotechnology companies, as well as investment banks and venture capital firms. Mark is often quoted in many industry publications for his insights into the IVD industry.

Mark began his career in clinical research at the Harvard School of Public Health and progressed into immunoassay research and development for the former Dade Behring's Clinical Assay division. He has held management positions in market research and business planning at the Ares-Serono Group and Gene-Trak Systems. Mark received his B.S. from the University of Massachusetts at Amherst and his M.B.A. from Duke University.

Troy Galloway, President

Troy is a 30+ year veteran of the diagnostics industry with experience in global marketing, strategy development and business management along with a broad knowledge of the IVD industry and products. She understands how companies approach decision-making and the kinds of insights needed to successfully develop, design, and commercialize new technologies.

Since joining EAC in 2016, Troy has leveraged her expertise to assist clients with product development and pricing strategies, outcome assessments, acquisitions, head-to-head performance studies and market segmentation.



Troy has a BS in Medical Technology from the University at Albany and a MS in Business from Johns Hopkins University.