8-1-2018

Does Use of a Molecular Rapid Pathogen Kit Improve Outcomes in the Bacteremic and Critically Ill?

Julie P. Gedalecia

DOES USE OF A MOLECULAR RAPID PATHOGEN KIT IMPROVE OUTCOMES IN THE BACTEREMIC AND CRITICALLY ILL?

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of
Master of Medical Science

August 2018

Julie P. Gedalecia, PA-SII
Class of 2018
Yale Physician Associate Program

Matthew Grant, MD
Assistant Professor of Medicine
Leader, IM Residency Inpatient Curriculum Project
Yale School of Medicine

Table of Contents

LIST OF TABLES III

LIST OF FIGURES IV

ABSTRACT V

CHAPTER 1: INTRODUCTION 1

1.1 BACKGROUND 1
1.2 STATEMENT OF PROBLEM 3
1.3 GOALS AND OBJECTIVES 5
1.4 HYPOTHESIS 5
1.5 DEFINITIONS 6

CHAPTER 2: REVIEW OF THE LITERATURE 9

2.1 INTRODUCTION 9
2.2 REVIEW OF SEPSIS AND SEPTIC SHOCK DIAGNOSIS 9
2.3 REVIEW OF SEPTIC PATIENT POPULATIONS 13
2.4 REVIEW OF MOLECULAR RAPID DIAGNOSTIC TESTS 14
2.5 REVIEW OF ANTIMICROBIAL STEWARDSHIP PROGRAMS 22
2.6 SUMMARY OF THE PROPOSED STUDY 23

CHAPTER 3: METHODS 28

3.1 STUDY DESIGN 28
3.2 STUDY POPULATION AND SAMPLING 28
3.3 SUBJECT PROTECTION AND CONFIDENTIALITY 28
3.4 RECRUITMENT AND ENROLLMENT 30
3.5 STUDY VARIABLES AND OUTCOME MEASURES 30
3.6 DATA COLLECTION 31
3.7 SAMPLE SIZE CALCULATION 32
3.8 DATA ANALYSIS 32
3.9 TIMELINE AND RESOURCES 33

CHAPTER 4: CONCLUSION 36

4.1 ADVANTAGES AND DISADVANTAGES 36
4.2 CLINICAL AND PUBLIC HEALTH SIGNIFICANCE 37

APPENDIX A: ADULT CONSENT FORM 40

AUTHORIZATION 45

APPENDIX B: SAMPLE SIZE CALCULATION 46

BIBLIOGRAPHY 48

List of Tables

Table 1. Systemic Inflammatory Response Syndrome (SIRS) 10
Table 2. Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score 12
Table 3. Accelerate Pheno™ ID Panel 19
Table 4. Accelerate Pheno™ Antibiotic Susceptibility/Resistance Panel 20

List of Figures

Figure 1. Timeline of Accelerate Pheno™ vs standard technique 4

Abstract

Sepsis is a pervasive condition that carries a tremendous burden of disease in the form of financial cost, morbidity, and mortality. Culturing methods slow clinicians' ability to begin focused treatment, and increased antimicrobial resistance only intensifies the need for improved diagnostic tools. Rapid molecular diagnostic tests can shorten time to identify organisms, reduce inappropriate antibiotic treatment, and improve patient outcomes. A newly approved test has proven fast and accurate for identification and susceptibility, but has not been studied in regard to clinical outcomes. **Our study will compare the effect of the Accelerate™ system versus standard identification and susceptibility tests on patient length of stay.** In a randomized controlled trial, we will use the Accelerate Pheno™ system to identify microbes and drug-resistance in septic critical care patients. We expect that diagnosis using this test will result in faster, more focused therapy, which will shorten hospital stays and save lives.

Chapter 1: Introduction

1.1 Background

Sepsis is a critical illness that has plagued humankind for millennia, with literary references to its destructive course reaching as far back as Homer's *Iliad*, Hippocrates' *Corpus Hippocratum*, and the writings of Galen.[1] Defined as, "life-threatening organ dysfunction caused by a dysregulated host response to infection,[2]" sepsis continues to cause tremendous suffering, despite our best efforts to understand and treat it. Sepsis does not discriminate on the basis of geographical location, socio-economic status, or racial differences and can strike both the octogenarian and the newborn. The worldwide mortality rate for patients with sepsis is a staggering 35.3%.[3] The most severe manifestation of sepsis—septic shock—causes cellular, circulatory, and metabolic dysfunction that can increase mortality upward of 40%.[2,4] In the United States, infectious disease is one of the ten leading causes of mortality, and more people die in the intensive care unit (ICU) from sepsis than from heart attack, stroke, congestive heart failure, or acute respiratory failure.[5-8]

Despite the history and prevalence of sepsis, there is no single diagnostic test with which to diagnose it. Instead, providers must draw conclusions from a combination of clinical, radiological, laboratory, and microbiological findings.[2] Identification of the pathogen is a crucial yet time-consuming step in this process that is necessary to inform our choice of antimicrobial treatment. For patients who develop sepsis, time to effective therapy is of the essence, particularly in cases of septic shock, where it has been shown that mortality increases 7.6% for every hour that appropriate antibiotic treatment is

delayed.[9] For patients infected with multidrug-resistant organisms (MDROs), failure to find a timely treatment can be fatal.[10-12] And while the pharmaceutical world races to stay ahead of these "super bugs" by developing ever-stronger antimicrobials, the bugs are gaining ground.

Less than a year after penicillin was introduced into practice, four penicillin-resistant *Staphylococcus* strains were found in patients receiving the new antibiotic.[13] Antimicrobial resistance is now a presence in every country, and the World Health Organization has declared it a serious threat to global public health.[14] Misuse of antibiotics in agriculture and human medicine has only accelerated the rate of bacterial resistance.[14,15] For example, between 2010 and 2012, the incidence rate of carbapenem-resistant *Klebsiella* species in United States hospitals jumped from 1.6% to 10.4%.[16] And while the spread of MDROs was historically limited to hospital settings, it is now seen as an emerging community-acquired threat as well.[17] Over 2 million illnesses and approximately 23,000 deaths are attributed to antibiotic resistance each year in this country.[18] MDROs inflict an enormous financial burden as well. In the US, resistant organisms cost the healthcare system an additional $21-34 billion annually.[17]

In order to identify (ID) pathogens that cause blood stream infections (BSIs), a sample of blood must be incubated, monitored for microbial growth, and then plated. Antimicrobial susceptibility tests (ASTs) are then performed to determine appropriate treatment.[19] It can take 24-72 hours for a blood culture sample to turn positive for growth, and another 24-48 hours to determine antimicrobial susceptibility using traditional methods.[19] Rapid diagnostic tests using various molecular techniques involving DNA amplification/hybridization, nucleic acid probes, magnetic resonance, and mass

spectrometry have been developed to shorten time to identification of pathogens as well as resistance.[15,20] Studies have shown that these products have the potential to improve outcomes regarding mortality, length of stay, and lower healthcare costs.[21]

1.2 Statement of Problem

Despite many diagnostic products now available for rapid identification of blood borne microbes and drug susceptibility, no single method has shown sufficient sensitivity or specificity to identify all sepsis-related microorganisms.[21] Furthermore, there have been few prospective, randomized clinical trials that directly compare rapid diagnostics to blood cultures, particularly with respect to clinical outcomes.[21]

In February 2017, the FDA approved a new diagnostic test, the Accelerate Pheno™ system (AxDx), designed to rapidly identify the most common pathogens associated with sepsis. Using a blood culture sample, AxDx can provide ID and AST results in approximately 7 hours, one to two days faster than conventional culture and susceptibility methods (**Fig. 1**).[22,23] AxDx can recommend a minimum inhibitory concentration (MIC) based on morphokinetic response in the AST phase. The system also has the ability to confirm that a culture is monomicrobial and to test for multiple organisms in a polymicrobial sample.[24]

To date, there have been no prospective, randomized clinical trials comparing AxDx to standard lab methods in relation to clinical outcomes. With this study we intend to show an improvement in patient outcomes using diagnostic information gained from AxDx to guide more immediate and appropriate treatment for sepsis.

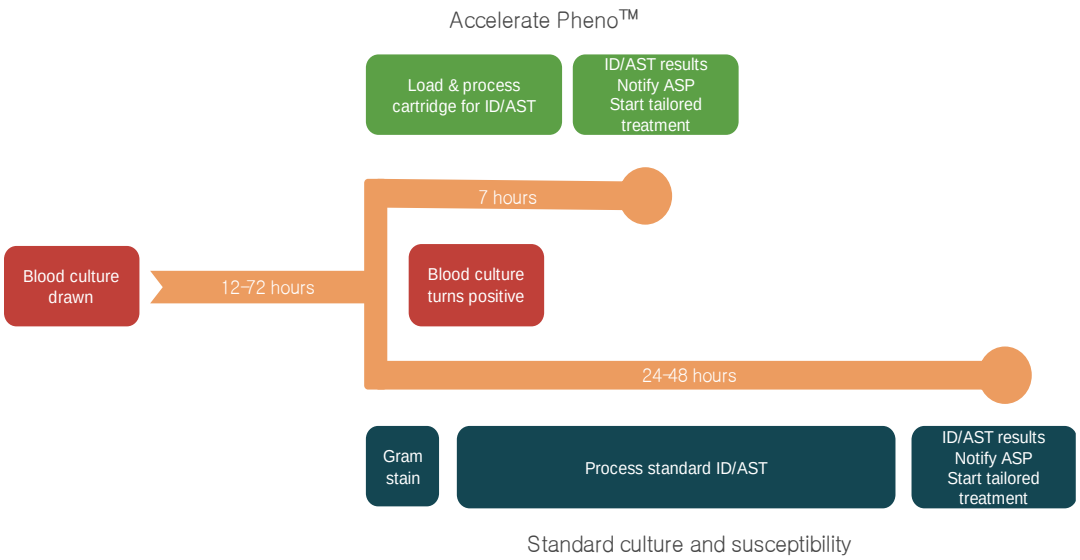

Figure 1.
Timeline of Accelerate Pheno™ vs standard technique

1.3 Goals and Objectives

The goal of this randomized controlled trial is to determine whether the use of AxDx to guide treatment will reduce length of stay in the intensive care unit (ICU LOS) for patients with sepsis, when compared to the use of standard laboratory methods. Along with our primary outcome, we will investigate effects on mortality, hospital length of stay (HLOS), and time to appropriate treatment (TAAT). To achieve the aforementioned goals of this study, we propose the following objectives:

I. Incorporate the AxDx into laboratory flow.

II. Emphasize current definitions and diagnostic criteria for sepsis and septic shock in the ICU.

III. Establish coordinated communication of results between microbiology department, antimicrobial stewardship team, and medical providers.

IV. Gain information on our patient population in terms of demographics, healthcare setting, source of infection, comorbidities, bacterial community composition, and antibiotic resistance.

1.4 Hypothesis

ICU LOS (measured as mean number of days) will be significantly different in septic ICU patients whose treatment is guided by AxDx run concurrently with standard culturing compared to those treated based on standard culturing alone.

1.5 Definitions

Antimicrobial resistance: the ability of microbes to resist the effects of drugs, allowing them to multiply and pass these traits on to others.

Antimicrobial stewardship program (ASP): clinicians, pharmacists, and staff with expertise in infectious disease, who oversee selection and dosing of antimicrobial therapy in order to minimize adverse reactions and reduce potential for antimicrobial resistance.[25]

Antimicrobial susceptibility test (AST): microbes are exposed to various drugs to determine which will be most effective in treating an infection.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF): Molecular process wherein a microbial colony is mixed with a matrix and irradiated by laser, thus becoming electrically charged and vaporized. An analyzer separates the molecules and compares their flight pattern to a database for species identification.[26]

Minimum inhibitory complex (MIC): the lowest concentration of an antimicrobial agent that will inhibit visible growth of an organism.

Peptide nucleic acid fluorescence in situ hybridization (PNA FISH): Molecular process using genetic probes labeled with fluorescent dye. The probe binds to a particular nucleic acid sequence on a microbial chromosome allowing visual identification of microbial species.[27]

Polymerase Chain Reaction (PCR): molecular technique using primers to locate specific nucleic acid sequences, which are then multiplied exponentially for species identification. PCR can be multiplexed, allowing more than one species in a sample to be identified.[28]

References

1. Funk DJ, Parrillo JE, Kumar A. Sepsis and septic shock: a history. *Critical care clinics.* 2009;25(1):83-101, viii.
2. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA.* 2016;315(8):801-810.
3. Vincent JL, Marshall JC, Namendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *The Lancet Respiratory medicine.* 2014;2(5):380-386.
4. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *Bmj.* 2016;353:i1585.
5. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *JAMA.* 2017;318(13):1241-1249.
6. Johnson NB, Hayes LD, Brown K, Hoo EC, Ethier KA. CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors--United States, 2005-2013. *MMWR supplements.* 2014;63(4):3-27.
7. Liu V, Escobar GJ, Greene JD, et al. Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA.* 2014;312(1):90-92.
8. Walker T, Dumadag S, Lee CJ, et al. Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures. *J Clin Microbiol.* 2016;54(7):1789-1796.
9. Banerjee R, Humphries R. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae. *Virulence.* 2017;8(4):427-439.
10. Girometti N, Lewis RE, Giannella M, et al. Klebsiella pneumoniae bloodstream infection: epidemiology and impact of inappropriate empirical therapy. *Medicine.* 2014;93(17):298-309.
11. Savage RD, Fowler RA, Rishu AH, et al. The Effect of Inadequate Initial Empiric Antimicrobial Treatment on Mortality in Critically Ill Patients with Bloodstream Infections: A Multi-Centre Retrospective Cohort Study. *PLoS One.* 2016;11(5):e0154944.
12. Chopra T, Marchaim D, Johnson PC, et al. Risk factors for bloodstream infection caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: A focus on antimicrobials including cefepime. *Am J Infect Control.* 2015;43(7):719-723.
13. Kong KF, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica.* 2010;118(1):1-36.
14. World Health Organization. Antimicrobial resistance: global report on surveillance 2014. Geneva: WHO; 2014. ISBN 9789241564748.

15. Maurer FP, Christner M, Hentschke M, Rohde H. Advances in Rapid Identification and Susceptibility Testing of Bacteria in the Clinical Microbiology Laboratory: Implications for Patient Care and Antimicrobial Stewardship Programs. *Infectious disease reports.* 2017;9(1):6839.
16. Exner M, Bhattacharya S, Christiansen B, et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS hygiene and infection control.* 2017;12:Doc05.
17. van Duin D, Paterson DL. Multidrug-Resistant Bacteria in the Community: Trends and Lessons Learned. *Infectious disease clinics of North America.* 2016;30(2):377-390.
18. Hampton T. Report reveals scope of us antibiotic resistance threat. *JAMA.* 2013;310(16):1661-1663.
19. Bhattacharya S. Early diagnosis of resistant pathogens: how can it improve antimicrobial treatment? *Virulence.* 2013;4(2):172-184.
20. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. *The Lancet Infectious diseases.* 2004;4(12):751-760.
21. Riedel S, Carroll KC. Early Identification and Treatment of Pathogens in Sepsis Molecular Diagnostics and Antibiotic Choice. *Clinics in Chest Medicine.* 2016;37(2):191-+.
22. Charnot-Katsikas A, Tesic V, Love N, et al. Use of the Accelerate Pheno System for Identification and Antimicrobial Susceptibility Testing of Pathogens in Positive Blood Cultures and Impact on Time to Results and Workflow. *J Clin Microbiol.* 2018;56(1).
23. Lutgring JD, Bittencourt C, McElvania TeKippe E, Cavuoti D, Hollaway R, Burd EM. Evaluation of the Accelerate Pheno System: Results from Two Academic Medical Centers. *J Clin Microbiol.* 2018;56(4).
24. Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S. Evaluation of the Accelerate Pheno System for Fast Identification and Antimicrobial Susceptibility Testing from Positive Blood Cultures in Bloodstream Infections Caused by Gram-Negative Pathogens. *J Clin Microbiol.* 2017;55(7):2116-2126.
25. Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(10):e51-77.
26. Tuma RS. MALDI-TOF Mass Spectrometry: Getting a Feel for How It Works. *Oncology Times.* 2003;25(19):26.
27. Stender H. PNA FISH: an intelligent stain for rapid diagnosis of infectious diseases. *Expert review of molecular diagnostics.* 2003;3(5):649-655.
28. Erlich HA. Polymerase chain reaction. *Journal of clinical immunology.* 1989;9(6):437-447.

Chapter 2: Review of the Literature

2.1 Introduction

This study will investigate whether the use of a new molecular rapid diagnostic test (mRDT), Accelerate Pheno™ (AxDx), to identify infectious pathogens will ultimately impact patient outcomes in the ICU. A comprehensive literature review was undertaken to identify studies comparing clinical outcomes of rapid tests to current standard microbiology methods. We searched PubMed, Embase, SCOPUS, Google Scholar, and Web of Science from August 2017-August 2018 for the following keywords and phrases: sepsis, severe sepsis, septic shock, septicemia, bacteremia, fungemia, rapid molecular diagnostic test, matrix-assisted laser desorption/ionization time-of-flight or MALDI-TOF, polymerase chain reaction or PCR, peptide nucleic acid fluorescence in situ hybridization or PNA FISH, antimicrobial resistance, antimicrobial stewardship, length of stay, mortality, and clinical outcomes. Randomized controlled trials, prospective and retrospective cohort trials, observational studies, consensus statements, conference publications, meta-analyses, and systematic reviews were included in the search. Articles from peer-reviewed sources were reviewed by title and abstract for relevancy. Articles referenced within these studies were also considered. The following represents a review of key studies examining the relationship under investigation.

2.2 Review of Sepsis and Septic Shock Diagnosis

In order to show the potential benefits of AxDx, we first had to assess the best method for identifying a septic population in the ICU. Despite our long-standing familiarity with this condition, the scientific community continues to debate over the defining characteristics of sepsis and the best clinical criteria for diagnosis. In the past 30

years, leaders in the infectious disease community have developed multiple iterations of the defining features of sepsis and how to approach it clinically.[1-3]

In 2016, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) task force scrutinized the 2001 definition[3] of sepsis, as well as previous diagnostic clinical criteria.[4] They concluded that sepsis be defined as, "life-threatening organ dysfunction caused by a dysregulated host response to infection."[4] The Sepsis-3 task force emphasized that, although sepsis-induced organ dysfunction may not manifest clinically, sepsis should be suspected in any patient with infection. Conversely, newly observed organ dysfunction may be the only clinical sign of underlying infection.

In 1992, the criteria for systemic inflammatory response syndrome (SIRS)—the clinical manifestation of immune response to inflammation—were recommended as the most effective method of identifying sepsis.[5] Meeting two or more of the SIRS criteria (**Table 1**) was an indication of developing severe sepsis. Sepsis-3, however, no longer recommends its application in this context.[4] Sepsis triggers both inflammatory and non-inflammatory responses, along with non-immunologic processes affecting cardiovascular, autonomic, metabolic, and neuronal pathways, and the task force concluded that SIRS did not sufficiently address our greater understanding of the condition.

Table 1. Systemic Inflammatory Response Syndrome (SIRS)

Two or more of the following:			
Temperature >38°C or <36°C	Heart rate >90/min	Respiratory rate >20/min or $PaCO_2$ <32 mm Hg	White blood cells >12000/mm^3 or <4000/mm^3 or >10% immature bands

Adapted from Bone et al.[2]

Kaukonen et al (2015), in an observational study conducted over 14 years at 172 ICUs in Australia and New Zealand, found that 1 in 8 patients with severe sepsis were missed using SIRS criteria.[6] These patients, 12.1% of the study population, suffered significant morbidity and mortality despite meeting fewer than 2 SIRS criteria. In a follow-up study, sparked by dissent from SIRS supporters[7], Kaukonen et al (2018) conducted another large study of >130,000 septic patients that revealed alarming disparity between various combinations of SIRS criteria as they relate to mortality.[8] For example, patients who met 2 SIRS criteria, high respiratory rate and high temperature, had a mortality rate of 11.5%, while patients with the same SIRS score due to low WBC and high heart rate had a 30.8% mortality. Similarly, patients with only low WBC (SIRS score of 1) had a 20.0% mortality rate, which was higher than 88% of the patients with SIRS scores of 2. These findings point to the fallibility of using any two SIRS criteria as a definitive marker for sepsis and bring into question its use in clinical practice as well as research studies.

As an alternative to SIRS, the Sepsis-3 task force determined that sepsis diagnoses be based on clinical impression and Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score ≥2 (**Table 2**).[4] In their evaluation of sepsis criteria validity, Seymour et al (2016) found that, in ICUs, the predictive validity of SOFA for hospital mortality was statistically greater (AUROC=0.74 [95% CI, 0.73-0.76]) than SIRS (AUROC=0.64 [95% CI, 0.62-0.66]; $P < .001$).[9] Cases were categorized into deciles of baseline risk. Patients in the ICU with ≥2 SOFA score saw a 3- to 11-fold increase in mortality depending on risk decile compared to those with ≥2 SOFA score, while those with ≥2 SIRS criteria saw only a 1- to 2-fold increased rate of mortality compared to

those with ≥2 SIRS criteria. Likewise, in a retrospective cohort analysis of 184,875 patients admitted to ICUs in Australia and New Zealand, Raith et al (2017) reported that SOFA criteria demonstrated a more accurate prognosis of outcomes than SIRS. [10] Patients with ≥2 SOFA score had a significantly incremental increase in risk of longer ICU stay (>3 days) and mortality at all deciles of baseline risk than those meeting ≥2 SIRS criteria.

Table 2. Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score

	1 point	2 points	3 points	4 points
Respiration PaO_2/FiO_2, mm Hg	<400	<300	<200	<100
			with respiratory support	
Coagulation Platelets, ×103/μL	<150	<100	<50	<20
Liver Bilirubin, mg/dL (μmol/L)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular	MAP <70 mm Hg	DA <5 or dobutamine (any dose)	DA 5.1-15 or EPI ≤0.1or NE ≤0.1*	DA >15 or EPI >0.1 or NE >0.1*
CNS Glasgow Coma Scale	13-14	10-12	6-9	<6
Renal Creatinine, mg/dL (μmol/L) or urine output	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440) or <500mL/day	>5.0 (440) or <200mL/day

Abbreviations: CNS, central nervous system, DA, dopamine, EPI, epinephrine, GCS, FiO_2, fraction of inspired oxygen, MAP, mean arterial pressure, NE, norepinephrine, PaO_2, partial pressure of oxygen
*Adrenergic agents dosed in μg/kg/min for at least 1 hour

Adapted from Vincent et al.[11]

Our review of the literature supports SOFA as a highly effective tool for evaluating septic patients in the ICU. The choice to use it as inclusion criteria will allow us to identify those most at risk for lengthy hospital stays and higher mortality.

2.3 Review of Septic Patient Populations

Blood stream infection (BSI) presents with a wide spectrum of severity, from transient, self-resolving illness to profound inflammatory response that leads to high rates of morbidity and mortality.[4] In order to effectively power our study of AxDx, the following review was focused on identifying the population most at risk for poor outcomes due to BSI. A retrospective cohort study by Rhee et al (2017), reviewing nearly 3 million adult electronic health records (EHRs) at 409 US hospitals, used the updated Sepsis-3 criteria of ≥2 SOFA score to identify patients with sepsis. Within this population they found a mean ICU LOS of 6.4 days (SD = 8.8), a mean HLOS of 12.0 days (SD = 12.1), and an estimated national in-hospital mortality rate of 15.6% (95% CI, 14.8-16.5).[12] A retrospective EHR review by Novosad et al (2016) was performed for the Centers for Disease Control "Morbidity and Mortality Weekly Report" using the discharge codes for sepsis recommended by Sepsis-3.[4,13] Of the 246 adult EHR from 4 different New York hospitals, 26% of adults with sepsis died, 6% were discharged to hospice, and the median HLOS was 9 days (ICU LOS was not assessed). Another retrospective study of 2.5 million cases stratified historic diagnoses of sepsis, severe sepsis, and septic shock according to the new Sepsis-3 guidelines.[4,14] Those with discharge codes corresponding with the task force criteria of ≥2 SOFA score were classified as septic. The study revealed mean HLOS of 10 days (SD = 12.4) and mean ICU LOS of 6.2 days (SD = 8.1). Mortality rates for sepsis were 14.9%.[14]

A study by Lilly et al (2017) looking at trends from 160 ICUs in the US found that the most common admission diagnosis was sepsis (8.5%), followed by respiratory failure (6.9%), acute coronary syndrome (6.9%), cardiac arrest (6.5%), cerebral vascular

accident (6.3%), GI bleed (5.4%), pneumonia (4.2%), trauma (4.1%), and congestive heart failure (4.0%).[15] Data from the most recent cohort (2013, N = 155,177) showed mean ICU LOS of 3.00 days (SD = 3.85) and 5.7% mortality. In the same year looking at the hospital population (N = 147,337), average HLOS was 7.42 days (SD = 7.21) days and mortality rate was 8.7%.

These findings show that not only does sepsis make up a significant portion of critical illness, but that sepsis patients spend much more time in the ICU than those with other serious conditions. On top of this they suffer from high rates of mortality, roughly 3 times greater than the average critical care patient. By selecting septic ICU patients who meet SOFA criteria, our study will target those who stand to benefit most from quick identification and treatment of infectious pathogens.

2.4 Review of Molecular Rapid Diagnostic Tests

In light of the significant morbidity and mortality caused by sepsis, the Infectious Diseases Society of America supports the use of rapid molecular diagnostic tests (mRDTs) and recognizes their potential to improve patient outcomes.[16] A variety of mRDTs have been developed to identify bacteria, viruses, and fungi faster than the current gold standard of conventional culture techniques. The three major categories of molecular techniques are PCR or other microarrays, PNA-FISH, and MALDI-TOF (definitions in Ch. 1). In a 2017 systematic review of 31 studies, Timbrook et al found that, in general, mRDT led to improvements in LOS, mortality, and time to appropriate antimicrobial treatment (TAAT).[17] However, the reviewers also found that some studies were not powered sufficiently to detect outcomes, while others were not designed to limit bias and reduce confounding. This limited their success in observing significant

differences between control and intervention and cast some doubt on their findings. Our review targeted studies examining patient outcomes, particularly in the ICU, when mRDTs were compared to standard lab methods. The most robust research to date on the aforementioned mRDT techniques is presented below.

The Verigene Blood Culture system (Luminex Corp.) is a multiplexed PCR assay that can detect twelve gram-positive bacterial species plus six resistance markers and nine gram-negative species plus three resistance markers directly from positive blood culture bottles.[18] Walker et al (2016) designed a quasi-experimental study comparing outcomes after ID and AST using Verigene for Gram-negative bacteria to standard ID and AST procedures.[19] An antimicrobial stewardship program (ASP) was present throughout the study. The assay was run on blood culture samples immediately after they signaled positive. Results showed significantly reduced ICU LOS, from 16.2 to 12.0 days (P = .03). The rate of 30-day overall hospital mortality decreased by over half (19.2% to 8.1%; P = 0.04). When analyzed with multivariate logistic regression, however, data showed a significant association between decreased 30-day mortality for ICU patients and the intervention (odds ratio, 0.81 [95% CI, 0.67-0.98]; P = .03). In cases of BSI caused by extended-spectrum beta-lactamase bacteria (ESBL), TAAT was reduced significantly from 41.4hr (SD = 9.0) to 7.3hr (SD = 9.0) (P = 0.04). In this quasi-experimental study, multi-drug resistant (MDR) bacteremia, including ESBL and carbapenem-resistant Enterobacteriaceae (CRE) sources, was associated with 12 of the 19 deaths in the control group compared to 1 of the 8 deaths in the Verigene group (P = .03). The study found no significant difference in HLOS or TAAT for other infections.

One limitation of this and many of the studies examined in our literature review is the quasi-experimental study design, typically performed in a pre- and post-intervention timeline. A principal problem inherent in these studies is lack of 1:1 randomization and, therefore, inability to control for confounding variables such as changes in hospital staff, microbial communities, and local resistance.[20] Due to the retrospective nature of Walker et al, one cannot assume that outcomes observed during the intervention period were a direct result of the use of Verigene.[19] Another limitation of this study was the evaluation of Verigene for gram-negative species only. While gram-negative species are associated with greater resistance and higher sepsis mortality rates (53.7% [SE = 0.3]), gram-positive bacteria still account for a considerable percentage of deaths in the US (42.3% [SE = 0.3]).[21] These limitations justify the need for a randomized controlled trial in which patients with BSI can be tested for the all of the common infectious causes of sepsis.

There are also considerable weaknesses in the design and application of Verigene. The system requires two different cassettes to process Gram-positive and Gram-negative samples.[18] Samples cannot be analyzed directly from blood culture bottles, but must first be Gram stained—a process performed by lab technicians that adds extra hands-on time. Another drawback of Verigene is its unreliability in detecting polymicrobial samples as well as identifying resistance genes.[22-24] In an assessment of Verigene by Bhatti et al (2014), the test failed to detect resistance based on the genotypic markers included in its screening panel.[24] The researchers discovered samples containing strains of *P. aeruginosa* that turned out to be phenotypically resistant. As stated by Maurer et al (2017), there is not always a direct correlation between the presence of a genetic resistance marker and phenotypic resistance.[25] This is why disk diffusion—where

resistance and susceptibility are exposed, regardless of genotype—remains the gold standard AST. The obvious drawback of this method is the 24-48 hour waiting period. An ideal mRDT would able to identify gram-positive, gram-negative, mixed infections, and also provide an accurate AST result in less time than the gold standard.

MALDI-TOF is another rapid diagnostic modality that has the ability to detect multiple organisms in a sample. Perez et al (2013) compared this method to conventional techniques in a quasi-experimental study at an academic hospital.[26] Adult patients were enrolled with gram-negative BSI confirmed by Gram stain, and clinical outcomes evaluated included LOS, mortality, and TAAT. An ASP was in place throughout the study. AST in the both groups was performed via BD Phoenix (Becton, Dickinson and Company). ICU LOS was 7.3 days (SD=8.5) for the control group compared to 6.3 days (SD=8.7) for the intervention (P = .05). ICU LOS from the onset of BSI, however, was not found to be significantly different (P = .09). Total HLOS and HLOS from onset of BSI were reduced significantly in the MALDI-TOF group, from roughly 12 to 9 days and 10 to 8 days respectively (P = .01 for both). TAAT in the control group was considerably slower at a mean 75hr (SD=48) compared to the mean 29hr (SD=17) in the intervention group (P = .004). After multivariate analysis, decreased HLOS was independently associated with the intervention (hazard ratio, 1.38 [95% CI, 1.01–1.88]) as well as appropriate antibiotic therapy at 48 hours (hazard ratio, 2.9 [95% CI, 1.15–7.33]).

While this study showed positive outcomes using MALDI-TOF, there are considerable weaknesses when comparing its methods and rapid test choice to our proposed research. While the MALDI-TOF method does allow for detection of multiple microbes in a sample, colonies must first be isolated. This involves bench time as well as

an incubation period. A limitation of Perez et al's design was enrollment at a single hospital and inclusion of only patients with monomicrobial, gram-negative BSI. They excluded any patients with gram-positive bacterial, fungal, or polymicrobial BSI. There was no difference in AST methods between the two arms of the study, which limits the potential effect of the rapid test. As mentioned earlier, mRDTs that include AST can cut an entire day off susceptibility wait times. The use of AxDx that provides both ID and AST in our study has the potential to show a greater difference in outcomes. Moreover, the inclusion of multiple hospital ICUs and septic patients with any type of BSI will power our study to demonstrate more significant differences in outcomes than those found by Perez and colleagues.

A thorough search for studies examining the use of PNA-FISH for BSI yielded mixed results. The majority of studies examining clinical outcomes were quasi-experimental or case-control designs examining PNA-FISH for a limited group of bacteria, i.e. only *Candida* spp. or coagulase-negative staphylococci.[27-32] The most robust study by Forrest et al (2008) compared a PNA-FISH test (AdvanDx, Inc.) for *Enterococcus* species, including *E. faecalis* and *E. faecium,* to conventional cultures.[28] The quasi-experimental, multi-center study enrolled all patients with blood cultures containing gram-positive cocci in pairs. An ASP was on-hand throughout the study. In patients with *E. faecium* bacteremia there was a reduction in 30-day mortality from 29% to 12% ($P = .039$) and a significant decrease in TAAT ($P < .001$) between control and intervention period. This study was limited by focusing on a very specific group of pathogens as well as its quasi-experimental design.

In February of 2017, the FDA approved AxDx, a new mRDT using FISH probes for genotypic ID and morphokinetic analysis for phenotypic susceptibility. AxDx is run directly on blood from positive blood culture bottles. Unlike previous PNA-FISH tests, AxDx has the ability to simultaneously target the most common sepsis-causing gram-positive and gram-negative bacteria, as well as two *Candida* species (**Table 3**).

Table 3. Accelerate Pheno™ ID Panel

Gram+ Bacteria	Gram- Bacteria	Fungi
Staphylococcus • *S. aureus* • *S. lugdunensis* • *S. capitis* • *S. epidermidis* • *S. haemolyticus* • *S. hominis* • *S. lugdunensis* • *S. warneri* ***Enterococcus*** • *E. faecalis* • *E. faecium* • *Enterococcus* spp. other than *E. faecalis* ***Streptococcus*** • *S. agalactiae* • *S. gallolyticus* • S. mitis • S. oralis • S. pneumoniae	***Klebsiella*** • *K. oxytoca* • *K. pneumoniae* *Enterobacter* • *E. cloacae* • *E. aerogenes* ***Proteus*** • *P. mirabilis* • *P. vulgaris* ***Citrobacter*** • *C. freundii* • *C. koseri* ***Other*** • *E. coli* • *S. marcescens* • *P. aeruginosa* • *A. baumannii*	***Candida*** • *C. albicans* • *C. glabrata*

Adapted from Accelerate Diagnostics, Inc.[33]

AxDx differs from the techniques mentioned above in that it utilizes digital microscopy to analyze individual cells and has the ability to identify one to four different

species in a single sample.[18] For AST it analyzes cell division, colony growth patterns, and morphological changes in response to antimicrobials using dark field microscopy and compares results to a compiled database.[18] From this information, AxDx delivers phenotypic AST as well as some phenotypic resistance results (**Table 4**). [18] As mentioned previously, phenotypic expression of resistance is a more reliable indicator of successful therapy than genetic markers, which are not always detected via molecular methods.[25]

Table 4. Accelerate Pheno™ Antibiotic Susceptibility/Resistance Panel

Phenotypic Susceptibility*		Phenotypic Resistance
• Ampicilin • Ceftaroline • Cefepime • Ceftazidime • Ceftriaxone • Erythromycin • Daptomycin • Linezolid • Vancomycin	• Ampicillin-Sulbactam • Piperacillin-Tazobactam • Ertapenem • Meropenem • Amikacin • Gentamicin • Tobramycin • Ciprofloxacin • Aztreonam	• Cefoxitin *S. Aureus* *S. lugdunensis* CNS spp. • Erythromycin/ Clindamycin *S. lugdunensis* CNS spp.

Adapted from Accelerate Diagnostics, Inc.[33]

AxDx is capable of processing samples from positive blood culture to ID and AST in approximately 7 hours. In a performance study by Marschal et al (2017), AxDx produced ID/AST results in approximately 8.88hr (IQR = 8.10-9.67), over 40hr faster than conventional ID/AST ($P < .001$).[34] AxDx cut AST time alone by approximately 27hr. The study focused on gram-negative agreement with conventional cultures and

found that AxDx correctly identified 102 of 105 monomicrobial samples (97.1%) and 10 of 10 polymicrobial samples (100%). Of the 13 incorrectly identified samples, 8 contained species not covered by the panel. AST agreement between AxDx and conventional cultures was 96.4%. They found 1.4% minor discrepancies 2.3%, major discrepancies 2.3%, and 1.0% very major discrepancies between methods. Of note, seven ESBL-producing *E. coli* and three MDR *P. aeruginosa* isolates were included in the evaluation, and AxDx correctly identified resistance and did not report false susceptibility.

In a comprehensive study by Pancholi et al (2017) for all pathogens covered by AxDx, a total of 1,940 samples were tested for ID/AST against standard lab methods.[35] AxDx provided an overall sensitivity of 97.5% (95% CI, 96.7-98.1) and specificity of 99.5% (95% CI, 99.4-99.5). A unique feature of AxDx is the ability to make a "monomicrobial call," wherein it can accurately determine if a sample contains only one species. When tested, the positive predictive value for monomicrobial samples was 97.3% (95% CI, 95.9- 98.2), and when missed samples were corrected using the accompanying Gram stain, the PPV rose to 99.4% (95% CI, 98.5-99.7).[35] This information could reassure providers that no further workup is necessary and result in a more rapid de-escalation of therapy.

A feature worth mentioning is AxDx's capacity to indicate if a detected species is not part of the regular panel, prompting further investigation in the lab. Another potential benefit of this system is the limited hands-on time required for operation. In a study by Charnot-Katsikas et al (2018), hands-on time was reduced by 25min on average compared to standard methods.[36]

These and other studies demonstrating the speed and accuracy of AxDx indicate the potential of this system to positively impact patient care.[36,37] With the possibility of reducing lab wait times by days and starting patients on appropriate therapy within hours, we could see significant improvements in BSI sepsis outcomes. Upon review of past studies investigating mRDTs and the limitations noted therein, a randomized, controlled clinical trial comparing AxDx to conventional methods as it pertains to patient outcomes is a logical and much needed next step.

2.5 Review of Antimicrobial Stewardship Programs

Along with the importance of identifying pathogens and their resistance to medications, an ASP's role in streamlining treatment for infectious disease is crucial.[16] By reducing the use of unnecessary antibiotics, an ASP can improve patient outcomes, ensure cost-effective therapy, and reduce adverse effects of broad-spectrum antibiotic use, most importantly antibiotic resistance.[16] Huang et al (2013) compared use of MALDI-TOF identification with antimicrobial susceptibility testing AST results before ASP implementation (control) and after ASP implementation (intervention) in a quasi-experimental study (N = 501).[38] Comparison of intervention to control showed a significant difference in ICU LOS (8.3 [SD = 24.2] vs 14.9 [SD = 9.0] days; P = .014), 30-day all-cause mortality (12.7% vs 20.3%; P = .021), faster time to effective therapy (20.4 vs 30.1 hours; P = .021), and faster time to optimal therapy (47.3 vs 90.3 hours; P < .001). It is important to note that there was no significant difference found in overall LOS and that possible confounding factors such as changes in standard of care, seasonal variations, or maturation bias of hospital staff could not be controlled.

Banerjee et al (2015) published a non-blinded, randomized, controlled trial comparing PCR mRDT with ASP (n = 212), without ASP (n = 198), and standard culturing (n = 207).[39] The study was not adequately powered to show differences in LOS, mortality, or hospital costs, but they found a significant difference in TAAT between mRDT with ASP and the other two arms. De-escalation to appropriate antibiotics in mRDT with ASP was 21hr, mRDT alone was 38hr, and control was 34hr ($P < 0.001$). Escalation to appropriate antibiotics in mRDT with ASP was 5hr, mRDT alone was 6hr, and control was 24hr ($P = .04$).

Upon review, it is clear that the information garnered from mRDT is more effectively understood and utilized when results are communicated through an ASP. Our findings support the Infectious Diseases Society of America's recommendation that ASP be used in conjunction with mRDT for BSI.[16] For our study, we feel it is imperative that the ASP works in close concert with both lab and clinician to interpret test findings and relay recommendations in a timely manner in order to maximize the benefits of using AxDx.

2.6 Summary of the Proposed Study

As of this writing there have been no RCTs comparing AxDx to conventional methods in regard to patient outcomes, although one such study is currently recruiting. Moreover, upon reviewing the current studies investigating mRDTs, the need for a randomized, controlled trial examining patient outcomes is clear. Many of the aforementioned studies, some more conclusively than others, showed improvement in patient outcomes with the use of mRDTs. They most commonly found reductions in ICU LOS, HLOS, TAAT, and, to a lesser degree, mortality. As our population of interest

includes only ICU patients, we are confident that choosing ICU LOS as our primary outcome will show the greatest measurable effect. Early and accurate diagnosis of sepsis is a key factor, and the use of SOFA in the ICU will help us identify patients most at risk for critical illness due to BSI. The participation of an ASP to guide treatment and track local resistance patterns has been proven to be a crucial part in the care and management of sepsis patients. Finally, with the use of AxDx, which has demonstrated high sensitivity, specificity, and considerably faster results than conventional methods, we hope to show significant differences in patient outcomes.

References

1. Balk RA, Bone RC. The septic syndrome. Definition and clinical implications. *Critical care clinics.* 1989;5(1):1-8.
2. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest.* 1992;101(6):1644-1655.
3. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med.* 2003;29(4):530-538.
4. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA.* 2016;315(8):801-810.
5. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Critical care medicine.* 1992;20(6):864-874.
6. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *The New England journal of medicine.* 2015;372(17):1629-1638.
7. Simpson SQ. SIRS in the Time of Sepsis-3. *Chest.* 2018;153(1):34-38.
8. Kaukonen K-M, Bailey M, Pilcher D, Cooper DJ, Bellomo R. The systemic inflammatory response syndrome criteria and their differential association with mortality. *Journal of Critical Care.* 2018;46:29-36.
9. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of clinical criteria for sepsis: For the third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA.* 2016;315(8):762-774.
10. Raith EP, Udy AA, Bailey M, et al. Prognostic Accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for In-Hospital Mortality Among Adults With Suspected Infection Admitted to the Intensive Care Unit. *JAMA.* 2017;317(3):290-300.
11. Vincent J-L, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Medicine.* 1996;22(7):707-710.
12. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *JAMA.* 2017;318(13):1241-1249.
13. Novosad SA, Sapiano MR, Grigg C, et al. Vital Signs: Epidemiology of Sepsis: Prevalence of Health Care Factors and Opportunities for Prevention. *MMWR Morbidity and mortality weekly report.* 2016;65(33):864-869.
14. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E. Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Critical care medicine.* 2018.
15. Lilly CM, Swami S, Liu X, Riker RR, Badawi O. Five-Year Trends of Critical Care Practice and Outcomes. *Chest.* 2017;152(4):723-735.

16. Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(10):e51-77.
17. Timbrook TT, Morton JB, McConeghy KW, Caffrey AR, Mylonakis E, LaPlante KL. The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2017;64(1):15-23.
18. Fairfax MR, Bluth MH, Salimnia H. Diagnostic Molecular Microbiology: A 2018 Snapshot. *Clinics in Laboratory Medicine.* 2018;38(2):253-276.
19. Walker T, Dumadag S, Lee CJ, et al. Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures. *J Clin Microbiol.* 2016;54(7):1789-1796.
20. Eliopoulos GM, Harris AD, Bradham DD, et al. The Use and Interpretation of Quasi-Experimental Studies in Infectious Diseases. *Clin Infect Dis.* 2004;38(11):1586-1591.
21. Ani C. Variations in organism-specific severe sepsis mortality in the United States: 1999-2008. *Critical care medicine.* 2015;43(1):65-77.
22. Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM. Evaluation of three rapid diagnostic methods for direct identification of microorganisms in positive blood cultures. *J Clin Microbiol.* 2014;52(7):2521-2529.
23. Suzuki H, Hitomi S, Yaguchi Y, et al. Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes. *Journal of Infection and Chemotherapy.* 2015;21(12):849-856.
24. Bhatti MM, Boonlayangoor S, Beavis KG, Tesic V. Evaluation of FilmArray and Verigene Systems for Rapid Identification of Positive Blood Cultures. *Journal of Clinical Microbiology.* 2014;52(9):3433-3436.
25. Maurer FP, Christner M, Hentschke M, Rohde H. Advances in Rapid Identification and Susceptibility Testing of Bacteria in the Clinical Microbiology Laboratory: Implications for Patient Care and Antimicrobial Stewardship Programs. *Infectious disease reports.* 2017;9(1):6839.
26. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. *Archives of pathology & laboratory medicine.* 2013;137(9):1247-1254.
27. Forrest GN, Mankes K, Jabra-Rizk MA, et al. Peptide nucleic acid fluorescence in situ hybridization-based identification of Candida albicans and its impact on mortality and antifungal therapy costs. *J Clin Microbiol.* 2006;44(9):3381-3383.
28. Forrest GN, Roghmann MC, Toombs LS, et al. Peptide nucleic acid fluorescent in situ hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. *Antimicrobial agents and chemotherapy.* 2008;52(10):3558-3563.

29. Ly T, Gulia J, Pyrgos V, Waga M, Shoham S. Impact upon clinical outcomes of translation of PNA FISH-generated laboratory data from the clinical microbiology bench to bedside in real time. *Therapeutics and clinical risk management.* 2008;4(3):637-640.
30. Koncelik DL, Hernandez J. The Impact of Implementation of Rapid QuickFISH Testing for Detection of Coagulase-Negative Staphylococci at a Community-Based Hospital. *American journal of clinical pathology.* 2016;145(1):69-74.
31. Forrest GN. PNA FISH: present and future impact on patient management. *Expert review of molecular diagnostics.* 2007;7(3):231-236.
32. Forrest GN, Mehta S, Weekes E, Lincalis DP, Johnson JK, Venezia RA. Impact of rapid in situ hybridization testing on coagulase-negative staphylococci positive blood cultures. *The Journal of antimicrobial chemotherapy.* 2006;58(1):154-158.
33. Accelerate Diagnostics. Accelerate PhenoTest™ BC Kit Panel. http://acceleratediagnostics.com/products/accelerate-phenotest-bc/ - panel.
34. Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S. Evaluation of the Accelerate Pheno System for Fast Identification and Antimicrobial Susceptibility Testing from Positive Blood Cultures in Bloodstream Infections Caused by Gram-Negative Pathogens. *J Clin Microbiol.* 2017;55(7):2116-2126.
35. Pancholi P, Carroll KC, Buchan BW, et al. Multicenter Evaluation of the Accelerate PhenoTest BC Kit for Rapid Identification and Phenotypic Antimicrobial Susceptibility Testing Using Morphokinetic Cellular Analysis. *J Clin Microbiol.* 2018;56(4).
36. Charnot-Katsikas A, Tesic V, Love N, et al. Use of the Accelerate Pheno System for Identification and Antimicrobial Susceptibility Testing of Pathogens in Positive Blood Cultures and Impact on Time to Results and Workflow. *J Clin Microbiol.* 2018;56(1).
37. Lutgring JD, Bittencourt C, McElvania TeKippe E, Cavuoti D, Hollaway R, Burd EM. Evaluation of the Accelerate Pheno System: Results from Two Academic Medical Centers. *J Clin Microbiol.* 2018;56(4).
38. Huang AM, Newton D, Kunapuli A, et al. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2013;57(9):1237-1245.
39. Banerjee R, Teng CB, Cunningham SA, et al. Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2015;61(7):1071-1080.

Chapter 3: Methods

3.1 Study Design

A prospective, randomized-controlled trial will be conducted at Yale New Haven Hospital for 24 consecutive months. Outcomes will be assessed for two groups receiving treatment based on diagnostic results from the Accelerate Pheno™ System versus standard culturing methods.

3.2 Study Population and Sampling

Eligible patients are adults (≥18 years) with SOFA scores greater than or equal to 2, who have positive blood culture results, and who are currently admitted to the following ICUs: medical (MICU), cardiac (CCU), cardiothoracic (CTICU), surgical (SICU) and neurocritical (NICU). Written informed consent will be procured from all patients or their power of attorney. If consent cannot be given during emergency situations, the FDA's guidelines on exceptions from informed consent will be followed.[1] Patients excluded meet the following criteria: (1) those with sepsis admitted to other units; (2) those with positive blood cultures in the preceding week; (3) those who decline participation; (4) those previously enrolled in the study; (5) those who die or are transferred off the unit within 24 hours of enrollment; or (6) those with positive cultures suspected to be contaminated by skin flora.

3.3 Subject Protection and Confidentiality

We will gain approval from Yale's Institutional Review Board (IRB) by satisfying all application requirements, providing a detailed study protocol, and eliciting

informed consent from subjects or power of attorney. The consent form (**Appendix A**) will outline the study in easily understood language, including risks and benefits of participation, as well as the exchange of personal health information (PHI) with the investigation team. We will follow all requirements for human subject research set forth by the Yale Human Research Protection Program.

All members of the investigation team will complete the human research requirements set forth by Yale, which include an initial web-based training program, Health Insurance Portability and Accountability Act (HIPPA) Training, and Good Clinical Practice Training prior to the start of the study. All information obtained during the study will be stored in an encrypted database. Select members of the research team will be allowed access to the data and only then by way of individual user IDs and passwords. University resources for data encryption (Bitlocker, FileVault), storage and document management (Secure Box, Storage@Yale), and a virtual private network (VPN) will be utilized in accordance with Yale Information Technology Services guidelines.

In order to protect patients and ensure ethical treatment, samples from the intervention group will also be processed using standard culturing and sensitivity methods. If additional blood samples are needed to accommodate this safeguard, they will be collected from all participants. Should a discrepancy between results arise, clinicians will make ultimate decisions regarding management and treatment.

3.4 Recruitment and Enrollment

During the 22-month rolling recruitment period, the research team will screen patients at YNHH for inclusion criteria. Once informed consent is given, participants will be enrolled in the study and randomized to either an intervention or a control group.

3.5 Study Variables and Outcome Measures

Assignment of intervention

Stratified randomization will be based on age (<65 or ≥65 years). Enrolled patients will be randomized 1:1 to either standard blood culture processing or Accelerate Pheno™ (AxDx). Patients will be blindly randomized and given a unique numeric identification code using most the current version of random allocation software.

Intervention and Control Variables

The independent variable will be the use of AxDx on positive blood culture samples. Specimens will be processed according to the manufacturer's specifications. Once the identity of microbial species and antimicrobial susceptibility are resulted, the ASP will be notified. ASP will interpret results and discuss appropriate treatment options with the patient's medical providers.

The active control group will be those whose samples are processed using standard YNHH culturing and sensitivity methods. Conveyance of results to ASP, as well as review and recommendations to providers, will follow the same protocol as in the intervention group. In both groups, providers will oversee the ordering and administration of treatment for their patients.

Dependent Variables

The primary outcome measured in this study will be ICU LOS, which will be measured quantitatively as a mean. A secondary outcome will be HLOS also measured as a mean. Other outcomes of interest to our investigators include 30-day mortality—measured as an incidence proportion of participants who die within 30 days of enrollment—and time to appropriate antimicrobial therapy (TAAT) measured quantitatively using means.

Blinding

Due to different laboratory methods between intervention and control, it will not be possible for lab personnel to be blinded to group assignments. Providers will not know group assignments at time of randomization so initial antibiotic therapy will be unaffected, but due to time-to-result differences between intervention and control, blinding of providers is not possible beyond enrollment. The research personnel reviewing electronic medical records (EMR) for outcomes of interest cannot be blinded to group assignments. Patients and investigators assessing outcome measures, however, will be blinded.

3.6 Data Collection

Baseline and Follow-up Data

Baseline demographic and clinical data of participants will be obtained at time of enrollment. Primary and secondary outcome measures will be assessed 30 days after enrollment. All information regarding hospital course will be retrieved from each participant's EMR. Research assistants will collect and compile data in the secured study

database and assign a computer-generated study identification number to each participant.

Confounding Data

Potential confounding variables that we plan to control for in this study include demographic information such as age, sex, and ICU location at enrollment. Clinical confounding factors include comorbidities, source of infection (if clinically evident), and severity of illness.

3.7 Sample Size Calculation

We used the statistics application PS: Power and Sample Size Calculation (version 3.1.2, Dale Plummer 2014) to compare two means for a two-sided hypothesis with a confidence level of 5% and power of 80%. We assumed an expected difference of effect measured as mean ICU LOS of 2 days with a standard deviation of 8.8. This resulted in a total sample size of 610 or 305 per arm. Factoring in a 1% attrition rate to each arm (lost to follow up prior to 30 days) results in a final sample size of 616 or 308 participants per arm. Details on sample size calculation can be found in Appendix B.

3.8 Data Analysis

Data collected during this study will be analyzed using statistical analysis software. Baseline characteristics of both groups collected at enrollment will be analyzed using standard parametric methods. To avoid confounding, continuous variables will be analyzed using Chi-square, while Student t-tests will be used to analyze categorical variables. The primary outcome of mean ICU LOS will be compared between intervention and control groups using Student t-test. Other outcomes such as mean

HLOS and mean TAAT will also be compared using Student t-tests. Incidence of 30-day mortality will be given as a percentage and proportion using Chi-square test. Simple logistic regression and multiple linear regression analyses will be used to control for significant differences in covariates between the groups at baseline.

3.9 Timeline and Resources

Our study will be carried out over 24 consecutive months at YNHH York Street Campus. Recruitment will be on a rolling basis throughout the first 22 months. Enrollment will end on the first day of month 22, allowing for the final participants to be followed for 30 days and for data collection and analysis to be performed over the final month.

Accelerate Diagnostic, Inc. will provide all funding necessary for this study, including appropriate number of Accelerate Pheno™ kits to accommodate specimen processing in the YNHH lab. Accelerate will also provide any necessary training to laboratory staff.

Study personnel will be include primary investigator and thesis advisor, Matthew Grant, MD, and student primary investigator, Julie Gedalecia, PA-SII. Research assistants will be required to relay questions and concerns from ICU and lab staff to the investigation team. They will perform data collection from EMR throughout the study and compile information into the database. A secured, dedicated office and computer will be necessary for data collection and analysis. A biostatistician will assist in developing randomization procedure for enrollment, as well as performing all statistical analyses. Cooperation from ICU sites will be necessary to coordinate recruitment and enrollment.

Reference

1. Guidance for Institutional Review Boards, Clinical Investigators, and Sponsors Exception from Informed Consent Requirements for Emergency Research. March 2011; https://www.fda.gov/RegulatoryInformation/Guidances/ucm122046.htm.

Chapter 4: Conclusion

4.1 Advantages and Disadvantages

Very few RCTs measuring patient outcomes in relation to mRDTs have been published. Ours would be the first RCT evaluating AxDx's clinical impact on patients in a head-to-head comparison with standard culture techniques. An advantage of our study design over previous research will be 1:1 randomization of participants, which will allow us to control for confounding factors. To reduce the chance of bias, our study participants will be blinded to assignments whenever possible, as explained in the methods section.

This study will be conducted at a large, urban academic hospital and may not be generalizable to smaller facilities in different geographical areas. Such smaller facilities might not have a round-the-clock ASP for consultations or laboratory space to implement new equipment. Regional rates of resistance can also vary greatly, and while Yale-New Haven Hospital has a relatively low incidence of carbapenem-resistant Enterobacteriaceae, hospitals in Boston, New York City, and Philadelphia see much higher rates (M. Grant, MD, oral communication, Aug 2018). BSIs and sepsis are not exclusive to the ICU, and studies will need to be conducted testing outcomes in populations on the floors and in the emergency department. Finally, our choice to exclude pediatric patients is based on their omission from the Sepsis-3 study.[1] The task force pointed out the need for updated criteria taking into account this population's "age-dependent variation in normal physiologic ranges and in pathophysiologic responses."

4.2 Clinical and Public Health Significance

The potential of AxDx to reduce TAAT by hours, if not days, could have a substantial, positive effect on patient outcomes. In a retrospective study of septic ICU patients with BSI conducted by Zhang et al (2017), TAAT was an independent determinant of attributable ICU LOS.[2] For every hour of appropriate treatment delay, there was a 0.095-day increase in ICU LOS from time of blood culture collection (95% CI, 0.057-0.132; $P < .001$). Likewise, every hour of delayed effective treatment saw a 0.134-day increase in attributable HLOS (95% CI, 0.074-0.194; $P < .001$). Initiation of appropriate treatment could result in patients suffering from fewer toxic events due to shorter periods of broad-spectrum antimicrobial treatment.[3] Studies have also shown that patients with long exposure to antimicrobials have an increased risk of acquiring secondary infections such as C. difficile.[4] From a stewardship standpoint, an expeditious switch from broad-spectrum antimicrobials to more tailored therapies can help reduce the spread of antimicrobial resistance.[5]

Although not addressed explicitly in our review, the fiscal implications of reducing morbidity and mortality from sepsis should not be overlooked. According to a comprehensive study by Paoli et al (2018), sepsis ranks highest of all disease states in management costs.[6] In the US in 2013, the $24 billion spent on sepsis care made up approximately 13% of all hospital costs. At roughly $18,000 per hospitalization, sepsis ranks a comfortable first place, before osteoarthritis at ~$16,000 per stay and childbirth at ~$3500 per stay. In another analysis of critical care in the US by Halpern and Pastores (2010), the daily cost of an ICU bed averaged $3500.[7] Despite our understanding of the

financial burden sepsis places on patients and the healthcare system, the practical question is whether or not the benefits of these new technologies outweigh the costs.

According to a representative from Accelerate, the list price of a system, which includes a computer used for analysis and two test modules that can process one sample each, is roughly $200,000. Each additional module is $80,000 with a maximum of four modules per computer system. The AxDx single-use, single-sample test cartridges cost roughly $200-250 each (phone communication, C. Peburn, Aug 2018). Theoretically, with an initial investment of $360,000 (one system with four modules), a hospital would recover these costs if ICU LOS decreased by one day for approximately 110 patients. According to national estimates of ICU admissions for sepsis (~14.6%)[8] and mean ICU LOS (6.4 days)[9], total cost from a system-wide perspective could be swiftly recuperated.

While AxDx promises to deliver a wealth of valuable information to providers and clinically beneficial outcomes to patients, these claims need to be evaluated in real-world settings. The results of our literature review and study will hopefully inspire further RCTs in this area. With the variety of mRDTs available and in development, sound research is needed in order to move us toward the next generation of diagnostic tests, as complications from serious blood-stream infections continue to exact an enormous toll on human health.

References

1. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA.* 2016;315(8):801-810.
2. Zhang D, Micek ST, Kollef MH. Time to Appropriate Antibiotic Therapy Is an Independent Determinant of Postinfection ICU and Hospital Lengths of Stay in Patients With Sepsis*. *Critical care medicine.* 2015;43(10):2133-2140.
3. Banerjee R, Teng CB, Cunningham SA, et al. Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2015;61(7):1071-1080.
4. Stevens V, Dumyati G, Fine LS, Fisher SG, van Wijngaarden E. Cumulative antibiotic exposures over time and the risk of Clostridium difficile infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2011;53(1):42-48.
5. Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(10):e51-77.
6. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E. Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Critical care medicine.* 2018.
7. Halpern NA, Pastores SM. Critical care medicine in the United States 2000-2005: an analysis of bed numbers, occupancy rates, payer mix, and costs. *Critical care medicine.* 2010;38(1):65-71.
8. Lilly CM, Swami S, Liu X, Riker RR, Badawi O. Five-Year Trends of Critical Care Practice and Outcomes. *Chest.* 2017;152(4):723-735.
9. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *JAMA.* 2017;318(13):1241-1249.

Appendix A: Adult Consent Form

COMPOUND AUTHORIZATION AND CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT
200 FR. 1 (2016-2)
YALE UNIVERSITY SCHOOL OF MEDICINE
YALE NEW HAVEN HOSPITAL

Study Title: Does Use of a Molecular Rapid Pathogen Kit Improve Outcomes in the Bacteremic and Critically Ill?
Study arms: Accelerate Pheno System ID/AST versus conventional culture and sensitivity
Principal Investigators: Julie Gedalecia, PA-SII, Matthew Grant, MD

Invitation to Participate and Description of Project

You are invited to participate in a research study designed to look at the comparison of a rapid diagnostic blood test versus standard blood test in the diagnosis and treatment of blood stream infection. You have been asked to participate because you are an ICU patient who has been diagnosed with sepsis by meeting two or more criteria (SOFA score) and having a blood culture that is positive for bacteria. This is a study is being carried out at Yale New Haven Hospital York Street Campus.

In order to decide whether or not you wish to be a part of this research study you should know enough about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research study, which a member of the research team will discuss with you. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, and any risks of the procedures, possible benefits, and possible alternative treatments. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Description of Procedures

If you agree to participate in this study, information from your medical records will be made available to the research team including age, sex, past medical history, past surgical history, medications, and allergies. Information specific to the study will be reported to the research team by lab personnel and your primary clinicians. This is done to compare the two groups to one another. All information is outlined in the following chart and will be reported at time of enrollment.

Medical information includes:

- Age, sex
- A measurement of your current level of disease known as the Sequential Organ Failure Assessment (SOFA)
- The reason you were admitted to the ICU

If you are an appropriate candidate for this study, you will be placed into either the intervention group or a control group; the intervention group has blood tested with the Accelerate system and the control group has blood tested with standard techniques. To date, there is no information supporting one test as superior to the other in terms of length of stay and survival. Random assignment occurs through a computer based system in which you have an equal chance of being placed in either the intervention or control group. Both you and your providers will be blinded to which test you receive, and all patients will be treated the same in terms of initial treatment for suspected blood infection. Once assigned to the group, you will be given an individualized study code to help maintain further privacy of your medical records.

We will be measuring the primary outcome of length of stay in the ICU along with three secondary outcomes. These outcomes will be reported at 30 days. 30 days from when you are entered into the study will mark the conclusion of the trial. You will continue your treatment and/or hospital stay if necessary but there will be no more information collected past this point. After the two-year study timeframe and data analysis, all identifying information will be deleted to maintain your privacy. Both test methods require the same amount of blood, approximately 140mL of blood, which is a little less than 5oz. All patients with suspected blood infection, not just those in this study, get this amount of blood drawn in our institution. Other outcomes are determined in respect to guidelines and clinical assessment, as listed below.

Other Measurements: 90-day survival**,** length of hospital stay, time to appropriate treatment

If you agree to participate in this study, you will be asked to:
- Consent to randomly being assigned to the Accelerate or standard group
- Consent to be blinded to test used
- Consent to allow access of the primary research team to your medical records, to obtain the information outlined above at the time of enrollment in the trial and up to 30 days after.
- Repeat blood draws may be necessary if results from either test are inconclusive

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.
You will be told of any significant new findings that are developed during the course of your participation in this study that may affect your willingness to continue to participate.

Risks and Inconveniences

Currently there is evidence that Accelerate is superior to standard technique in terms of patient outcomes. Accelerate has been measured against standard technique and has a significant level of agreement. This study potentially changes speed at which treatment is delivered, so there is some risk of being treated slower than the standard of care. However, since standard of care technique will be performed on all participants, should there be a failure of Accelerate to deliver a result, we will use the standard technique results to formulate a treatment plan. Since you are a patient in a critical care unit,c there are common risks associated with being in the ICU such as:

- Death
- Worsening of illness with possibility of the need for life support through mechanical ventilation or blood pressure supporting drugs
- Renal failure requiring renal replacement therapy such as dialysis
- Other organ failure
- Secondary infection

Additionally, blood tests have some minor risks associated with them such as swelling bruising, and bleeding, feeling lightheaded or nauseous, and rarely infection at the site from where blood was drawn. Our clinical team will take all necessary steps to maintain a sanitary environment and use proper technique to prevent infection and limit pain.

As we are collecting medical information, there is risk of loss of confidentiality. We are complying with all guidelines taking every step to ensure that your private information is secure. Identifying information will only be stored on a single online document at the primary research center, to be deleted following conclusion of the study.

Benefits

Participation in this study allows you to randomly be assigned to either Accelerate or standard technique as your blood test method. There may be the benefit that being assigned to the experimental intervention, Accelerate, yields a shorter length of stay or higher incidence of survival as compared to the standard technique. Furthermore, this study may help direct the way in which we test patients who have serious blood infections, its results having the potential to help many patients in future.

Economic Considerations

Regardless of randomization, both tests will be provided to you are at no cost. Adverse effects as a result of the blood tests will be treated at no cost as well. You will be responsible for any co-pays required by your insurance company for treatment of your illness.

Treatment Alternatives

If you decide not to participate in this study, you will be tested using standard technique.

Confidentiality
Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases.

Compliance information and compliance requirements mandated by Health Insurance Portability and Accountability Act (HIPAA) will be reviewed and employed in our study design. This specifically encompasses both cyber and physical security. Additionally, all subjects will be assigned individualized numbers to remove identifying factors; Patient identification numbers and information will be stored in a singular master list, remaining on a secure server that will be deleted following study completion after a 2-year study period and data analysis. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity unless your specific consent for this activity is obtained.

Representatives from the Yale Human Research Protection Program, the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects) may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

Information about your study participation will be entered into your Electronic Medical Record (EMR). Once placed in your EMR, these results are accessible to all of your providers who participate in the EMR system. Information within your EMR may also be shared with others who are appropriate to have access to your EMR (e.g. health insurance company, disability provider.)

In case of Injury
If you are injured while on study, seek treatment and contact the study doctor as soon as you are able.

Yale School of Medicine and Yale-New Haven Hospital do not provide funds for the treatment of research-related injury. If you are injured as a result of your participation in this study, treatment will be provided. You or your insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available.

You do not give up any of your legal rights by signing this form.

Voluntary Participation and Withdrawal

Participating in this study is voluntary. You are free to choose not to take part in this study. Refusing to participate will involve no penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits). However, you will not be able to enroll in this research study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study.

If you do decide to take part in this study, you are free to stop and withdraw from this study at any time during its course. Patients maintain the right to refuse the blood test. Once withdrawn from the trial, your illness treatment will be managed at the discretion of the primary clinical team.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part. The researchers may withdraw you from participating in the research if necessary. This may occur under the conditions that you are found to be participating in another clinical trial.

Withdrawing from the study will involve no penalty or loss of benefits to which you are otherwise entitled. It will not harm your relationship with your own doctors or with the hospital.

When you withdraw from the study, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to insure the integrity of the study and/or study oversight.

Questions

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the consent form carefully—as long as you feel is necessary—before you make a decision.

Authorization

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject:_____

Signature:_____

Date:_____

In the event the participant has impaired decision-making capacity, I have decided to allow participation in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Durable Power of Attorney for Health Care Name:_____

Signature:_____

Date:_____

_____ _____
Signature of Principal Investigator Date

-or-

_____ _____
Signature of Person Obtaining Consent Date

If you have further questions about this project or have a research-related problem, you may contact Principal Investigators, Julie Gedalecia, PA-SII or Dr. Matthew Grant, MD.

If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

Appendix B: Sample Size Calculation

In a previous study by Rhee et al. (2017)[1] reviewing nearly 3 million adult EHRs, the mean ICU LOS for patients with sepsis was 6.4 days (SD = 8.8). Our aim is to detect a mean change difference of 2 (SD = 8.8) between the groups in our study.

Sample size was calculated with PS: Power and Sample Size Calculations (version 3.1.2, Dale Plummer 2014) in August 2018.

Power (1-β)	0.80
Type 1 error (α)	0.05
Number of tails	2
Mean change	2.0
Standard Deviation	8.8

305 subjects per arm or 610 subjects total is the raw sample size.

Estimating for a 1% loss to follow-up, the final sample size is **308 subjects per arm or 616 total subjects.**

Reference

1. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *JAMA.* 2017;318(13):1241-1249.

Bibliography

1. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Critical Care Medicine.* 1992;20(6):864-874.
2. Guidance for Institutional Review Boards, Clinical Investigators, and Sponsors Exception from Informed Consent Requirements for Emergency Research. March 2011; https://www.fda.gov/RegulatoryInformation/Guidances/ucm122046.htm.
3. Accelerate Diagnostics. Accelerate PhenoTest™ BC Kit Panel. http://acceleratediagnostics.com/products/accelerate-phenotest-bc/#panel.
4. Ani C. Variations in organism-specific severe sepsis mortality in the United States: 1999-2008. *Critical Care Medicine.* 2015;43(1):65-77.
5. Balk RA, Bone RC. The septic syndrome. Definition and clinical implications. *Critical care clinics.* 1989;5(1):1-8.
6. Banerjee R, Humphries R. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae. *Virulence.* 2017;8(4):427-439.
7. Banerjee R, Teng CB, Cunningham SA, et al. Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2015;61(7):1071-1080.
8. Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(10):e51-77.
9. Bhattacharya S. Early diagnosis of resistant pathogens: how can it improve antimicrobial treatment? *Virulence.* 2013;4(2):172-184.
10. Bhatti MM, Boonlayangoor S, Beavis KG, Tesic V. Evaluation of FilmArray and Verigene Systems for Rapid Identification of Positive Blood Cultures. *Journal of clinical microbiology.* 2014;52(9):3433-3436.
11. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest.* 1992;101(6):1644-1655.
12. Charnot-Katsikas A, Tesic V, Love N, et al. Use of the Accelerate Pheno System for Identification and Antimicrobial Susceptibility Testing of Pathogens in Positive Blood Cultures and Impact on Time to Results and Workflow. *Journal of clinical microbiology.* 2018;56(1).
13. Chopra T, Marchaim D, Johnson PC, et al. Risk factors for bloodstream infection caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: A focus on antimicrobials including cefepime. *American journal of infection control.* 2015;43(7):719-723.

14. Eliopoulos GM, Harris AD, Bradham DD, et al. The Use and Interpretation of Quasi-Experimental Studies in Infectious Diseases. *Clinical Infectious Diseases.* 2004;38(11):1586-1591.
15. Erlich HA. Polymerase chain reaction. *Journal of clinical immunology.* 1989;9(6):437-447.
16. Exner M, Bhattacharya S, Christiansen B, et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS hygiene and infection control.* 2017;12:Doc05.
17. Fairfax MR, Bluth MH, Salimnia H. Diagnostic Molecular Microbiology: A 2018 Snapshot. *Clinics in Laboratory Medicine.* 2018;38(2):253-276.
18. Forrest GN. PNA FISH: present and future impact on patient management. *Expert review of molecular diagnostics.* 2007;7(3):231-236.
19. Forrest GN, Mankes K, Jabra-Rizk MA, et al. Peptide nucleic acid fluorescence in situ hybridization-based identification of Candida albicans and its impact on mortality and antifungal therapy costs. *Journal of clinical microbiology.* 2006;44(9):3381-3383.
20. Forrest GN, Mehta S, Weekes E, Lincalis DP, Johnson JK, Venezia RA. Impact of rapid in situ hybridization testing on coagulase-negative staphylococci positive blood cultures. *The Journal of antimicrobial chemotherapy.* 2006;58(1):154-158.
21. Forrest GN, Roghmann MC, Toombs LS, et al. Peptide nucleic acid fluorescent in situ hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. *Antimicrobial agents and chemotherapy.* 2008;52(10):3558-3563.
22. Funk DJ, Parrillo JE, Kumar A. Sepsis and septic shock: a history. *Critical care clinics.* 2009;25(1):83-101, viii.
23. Girometti N, Lewis RE, Giannella M, et al. Klebsiella pneumoniae bloodstream infection: epidemiology and impact of inappropriate empirical therapy. *Medicine.* 2014;93(17):298-309.
24. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ (Clinical research ed).* 2016;353:i1585.
25. Halpern NA, Pastores SM. Critical care medicine in the United States 2000-2005: an analysis of bed numbers, occupancy rates, payer mix, and costs. *Crit Care Med.* 2010;38(1):65-71.
26. Hampton T. Report reveals scope of us antibiotic resistance threat. *Jama.* 2013;310(16):1661-1663.
27. Huang AM, Newton D, Kunapuli A, et al. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2013;57(9):1237-1245.
28. Johnson NB, Hayes LD, Brown K, Hoo EC, Ethier KA. CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors--United States, 2005-2013. *MMWR supplements.* 2014;63(4):3-27.

29. Kaukonen K-M, Bailey M, Pilcher D, Cooper DJ, Bellomo R. The systemic inflammatory response syndrome criteria and their differential association with mortality. *Journal of Critical Care.* 2018;46:29-36.
30. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *The New England journal of medicine.* 2015;372(17):1629-1638.
31. Koncelik DL, Hernandez J. The Impact of Implementation of Rapid QuickFISH Testing for Detection of Coagulase-Negative Staphylococci at a Community-Based Hospital. *American journal of clinical pathology.* 2016;145(1):69-74.
32. Kong KF, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica.* 2010;118(1):1-36.
33. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive care medicine.* 2003;29(4):530-538.
34. Lilly CM, Swami S, Liu X, Riker RR, Badawi O. Five-Year Trends of Critical Care Practice and Outcomes. *Chest.* 2017;152(4):723-735.
35. Liu V, Escobar GJ, Greene JD, et al. Hospital deaths in patients with sepsis from 2 independent cohorts. *Jama.* 2014;312(1):90-92.
36. Lutgring JD, Bittencourt C, McElvania TeKippe E, Cavuoti D, Hollaway R, Burd EM. Evaluation of the Accelerate Pheno System: Results from Two Academic Medical Centers. *Journal of clinical microbiology.* 2018;56(4).
37. Ly T, Gulia J, Pyrgos V, Waga M, Shoham S. Impact upon clinical outcomes of translation of PNA FISH-generated laboratory data from the clinical microbiology bench to bedside in real time. *Therapeutics and clinical risk management.* 2008;4(3):637-640.
38. Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S. Evaluation of the Accelerate Pheno System for Fast Identification and Antimicrobial Susceptibility Testing from Positive Blood Cultures in Bloodstream Infections Caused by Gram-Negative Pathogens. *Journal of clinical microbiology.* 2017;55(7):2116-2126.
39. Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM. Evaluation of three rapid diagnostic methods for direct identification of microorganisms in positive blood cultures. *Journal of clinical microbiology.* 2014;52(7):2521-2529.
40. Maurer FP, Christner M, Hentschke M, Rohde H. Advances in Rapid Identification and Susceptibility Testing of Bacteria in the Clinical Microbiology Laboratory: Implications for Patient Care and Antimicrobial Stewardship Programs. *Infectious disease reports.* 2017;9(1):6839.
41. Novosad SA, Sapiano MR, Grigg C, et al. Vital Signs: Epidemiology of Sepsis: Prevalence of Health Care Factors and Opportunities for Prevention. *MMWR Morbidity and mortality weekly report.* 2016;65(33):864-869.
42. Pancholi P, Carroll KC, Buchan BW, et al. Multicenter Evaluation of the Accelerate PhenoTest BC Kit for Rapid Identification and Phenotypic Antimicrobial Susceptibility Testing Using Morphokinetic Cellular Analysis. *Journal of clinical microbiology.* 2018;56(4).

43. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E. Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Critical Care Medicine.* 2018.
44. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. *Archives of pathology & laboratory medicine.* 2013;137(9):1247-1254.
45. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. *The Lancet Infectious diseases.* 2004;4(12):751-760.
46. Raith EP, Udy AA, Bailey M, et al. Prognostic Accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for In-Hospital Mortality Among Adults With Suspected Infection Admitted to the Intensive Care Unit. *Jama.* 2017;317(3):290-300.
47. Rhee C, Dantes R, Epstein L, et al. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *Jama.* 2017;318(13):1241-1249.
48. Riedel S, Carroll KC. Early Identification and Treatment of Pathogens in Sepsis Molecular Diagnostics and Antibiotic Choice. *Clinics in Chest Medicine.* 2016;37(2):191-+.
49. Savage RD, Fowler RA, Rishu AH, et al. The Effect of Inadequate Initial Empiric Antimicrobial Treatment on Mortality in Critically Ill Patients with Bloodstream Infections: A Multi-Centre Retrospective Cohort Study. *PloS one.* 2016;11(5):e0154944.
50. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of clinical criteria for sepsis: For the third international consensus definitions for sepsis and septic shock (sepsis-3). *Jama.* 2016;315(8):762-774.
51. Simpson SQ. SIRS in the Time of Sepsis-3. *Chest.* 2018;153(1):34-38.
52. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama.* 2016;315(8):801-810.
53. Stender H. PNA FISH: an intelligent stain for rapid diagnosis of infectious diseases. *Expert review of molecular diagnostics.* 2003;3(5):649-655.
54. Stevens V, Dumyati G, Fine LS, Fisher SG, van Wijngaarden E. Cumulative antibiotic exposures over time and the risk of Clostridium difficile infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2011;53(1):42-48.
55. Suzuki H, Hitomi S, Yaguchi Y, et al. Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes. *Journal of Infection and Chemotherapy.* 2015;21(12):849-856.
56. Timbrook TT, Morton JB, McConeghy KW, Caffrey AR, Mylonakis E, LaPlante KL. The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2017;64(1):15-23.

57. Tuma RS. MALDI-TOF Mass Spectrometry: Getting a Feel for How It Works. *Oncology Times.* 2003;25(19):26.
58. van Duin D, Paterson DL. Multidrug-Resistant Bacteria in the Community: Trends and Lessons Learned. *Infectious disease clinics of North America.* 2016;30(2):377-390.
59. Vincent J-L, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive care medicine.* 1996;22(7):707-710.
60. Vincent JL, Marshall JC, Namendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *The Lancet Respiratory medicine.* 2014;2(5):380-386.
61. Walker T, Dumadag S, Lee CJ, et al. Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures. *Journal of clinical microbiology.* 2016;54(7):1789-1796.
62. World Health Organization. Antimicrobial resistance: global report on surveillance 2014. Geneva: WHO; 2014. ISBN 9789241564748.
63. Zhang D, Micek ST, Kollef MH. Time to Appropriate Antibiotic Therapy Is an Independent Determinant of Postinfection ICU and Hospital Lengths of Stay in Patients With Sepsis. *Critical Care Medicine.* 2015;43(10):2133-2140.