BAŞKENT UNIVERSITY
INSTITUTE OF SCIENCES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS
MOLECULAR BIOLOGY AND GENETICS MASTER'S PROGRAM

PERFORMANCE COMPARISON OF APTAMER AND ANTIBODY BASED FLUORESCENT BIOSENSORS FOR BACTERIA ON GLASS SURFACE

BY

ASLI KÜREKÇİ

MASTER OF SCIENCE THESIS

ANKARA – 2023

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This study, which was prepared by Aslı KÜREKÇİ within the framework of the Molecular Biology and Genetics Master's Program with Thesis in Molecular Biology and Genetics, was accepted as the Master's Thesis by the following jury.

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I would like to dedicate this work to my family for their unwavering love, encouragement, and support throughout this journey. Your belief in me has been a constant source of motivation, and I am grateful for your presence in my life.

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ABSTRACT

Aslı KÜREKÇİ

PERFORMANCE COMPARISON OF APTAMER AND ANTIBODY BASED FLUORESCENT BIOSENSORS FOR BACTERIA ON GLASS SURFACE

Başkent University Institute of Sciences

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Antibodies are the most commonly used ligands in commercial and research analysis systems to detect pathogenic cells. However, aptamers are superior ligands compared to antibodies and other preferred research molecules for developing sensitive and robust tests due to their small size, low cost, and easy chemical modification. Although aptamers offer many opportunities for developing molecular tools, comprehensive comparisons between aptamer-based biosensors and immunoassays are unfortunately limited to protein analytes. In this study, we present a comparison of the performance of antibody and aptamer ligands with the highest biosensor development potential on glass surfaces through systematic experiments for the pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii)* most commonly associated with sepsis in our country Turkey. The comparative study, conducted with a total of 12 ligands, reported superior success for anti-*E. coli* antibody (HRP) for *E. coli*, mouse monoclonal to *S. aureus* for *S. aureus*, and Elongation factor Tu polyclonal Antibody (tuf1) rabbit anti-*A. baumannii* for *A. baumannii*. The performance of the top-performing ligands was further validated through additional studies on the linearity, analytical sensitivity, and repeatability of the results. The results demonstrated that antibody ligands still outperform aptamer ligands in terms of efficiency, but aptamers continue to possess strong potential as an analytical tool.

KEYWORDS: Biosensor, bacteria detection, glass surface, aptamer, antibody

Tübitak 118E023 and 118E080 Support Programs, Denovo Biotechnology Company, Hacettepe University, Bilkent University, Başkent University

ÖZET

Aslı KÜREKÇİ

APTAMER VE ANTİKOR BAZLI FLORESAN BİYOSENSÖRLERİN CAM YÜZEYDEKİ BAKTERİLER İÇİN PERFORMANS KARŞILAŞTIRMASI

Başkent Üniversitesi Fen Bilimleri Enstitüsü

Moleküler Biyoloji Ve Genetik Programı

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Antikorlar, günümüzde patojen hücrelerini saptamak için ticari ve araştırma analiz sistemlerinde en yaygın olarak kullanılan ligandlardır. Buna karşın, aptamerler, küçük boyut, düşük maliyet ve kolay kimyasal modifikasyon gibi özellikleri nedeni ile hassas ve sağlam test geliştirmek için antikorlara ve diğer tercih edilen araştırma moleküllerine kıyasla avantajlı olan üstün ligandlardır. Aptamerler birçok moleküler araç geliştirme fırsatı sunmasına karşın, aptamer bazlı biyosensörler ve immünosensörler arasındaki kapsamlı karşılaştırmalar ne yazık ki sadece protein analitleriyle sınırlıdır. Bu çalışmada, ülkemizde sepsis hastalığına en çok neden olan patojenler (*Escherichia coli, Staphylococcus aureus* ve *Acinetobacter baumannii*) için sistematik deneylerle, cam yüzeylerde, biyosensör geliştirme potansiyeli en yüksek olan antikor ve aptamer ligandların performanslarının karşılaştırması sunulmuştur. Toplam 12 Ligand ile yapılan karşılaştırmalı çalışmanın sonucunda, *E. coli* için anti-*E. coli* antibody (HRP), *S. aureus* için mouse monoclonal *S. aureus* ve *A. baumannii* için Elongation factor Tu polyclonal Antibody (tuf1) rabbit anti-*A. baumannii* ligandlarının üstün başarı gösterdiği rapor edildi. En iyi performansa sahip ligandlar ile, sonuçların doğrusal aralığı, analitik duyarlılığı ve tekrarlanabilirliği için ayrıca çalışmalar yapılarak performansları doğrulandı. Sonuçlar, antikor ligandlarının aptamer ligandlarından daha yüksek bir verimlilikle işlev gördüğünü, ancak aptamerlerin analitik bir araç olarak güçlü bir potansiyele sahip olduğunu göstermiştir.

ANAHTAR KELİMELER: Biyosensör, bakteri tespiti, cam yüzey, aptamer, antikor

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PREFACE

It is with great pleasure that I present to you this thesis titled "PERFORMANCE COMPARISON OF APTAMER AND ANTIBODY BASED FLUORESCENT BIOSENSORS FOR BACTERIA ON GLASS SURFACE." This study aims to evaluate the performance of biosensor ligands, which play a crucial role in the detection of pathogens.

In today's world, the detection and control of diseases caused by microorganisms are of paramount importance. In this context, an efficient and reliable biosensor system can greatly facilitate this process by providing fast and accurate analysis methods. This study focuses on comparing the performance of aptamer and antibody-based ligands biosensors on glass surfaces. Aptamers, with their small size, low cost, and ease of chemical modification, have emerged as superior ligands for biosensors. However, antibodies have also proven themselves as widely used and established ligands for the detection of pathogenic cells. This study presents a comprehensive set of experiments to evaluate the performance of both aptamers and antibodies. The results of the study demonstrate which ligands perform better and important analytical features such as sensitivity, linear range, and repeatability. These findings will serve as valuable guidance for the development of biosensor technologies.

I would like to express my sincere gratitude to everyone who has contributed to the realization of this study. I especially want to thank my supervisor, Oğuz BALCI, for his guidance and support throughout the project. I would also like to extend my thanks to Denovo Biotechnology Inc. for their investment in the project and for providing the experimental environment. Furthermore, I would like to acknowledge Hacettepe University for presenting the project idea and providing the necessary samples. Special thanks go to Bilkent University for their support in conducting additional experiments. I would also like to extend my thanks to TÜBİTAK for their investment in the project. Lastly, I would like to express my deepest appreciation to my family for their unwavering support throughout this process.

I hope that this thesis provides valuable insights and contributes to the advancement of biosensor technology in the field of pathogen detection.

Sincerely,

ASLI KÜREKÇİ

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ABBREVIATIONS LIST

A. baumannii	*Acinetobacter baumannii*
BSI	Bloodstream Infections
C^0_{Apt}	Initial Aptamer Concentration
C^0_{Bac}	Initial Bacterial Concentration
C^0_{Pro}	Initial Antibody Concentration
C_{Apt}	Wash Aptamer Concentration
C_{Bac}	Wash Bacteria Concentration
CFU	Colony-Forming Unit
C_{Pro}	Wash Antibody Concentration
CRP	C-Reactive Protein
E. coli	*Escherichia coli*
ELISA	Enzyme-Linked Immunosorbent Assay
EOS	Early-Onset Sepsis
ESBL	Extended-Spectrum Beta-Lactamase
FISH	Fluorescence *In Situ* Hybridization
ICU	Intensive Care Unit
LOC	Lab-On-A-Chip
MALDI-TOF MS	Matrix-Assisted Laser Desorption-Ionization Flow Mass
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PDMS	Polydimethylsiloxane
S. aureus	*Staphylococcus aureus*
SELEX	Systematic Evolution of Ligands by Exponential Enrichment
UTIs	Urinary Tract Infections
WHO	World Health Organization

1. INTRODUCTION

One of the leading causes of illness and mortality known around the world is sepsis. In 2017, it had an impact on 48.9 million individuals and was the cause of 19.7% of all fatalities globally [1] . The primary causes for the low success rate of sepsis therapies are low consciousness, a lack of complete information about the pathophysiology of sepsis, difficulties in clinical diagnosis, and a lack of good prognostic indicators [2]. The diagnostic techniques now in use are based on conventional culturally based methodologies; they take a lot of time and postpone making crucial treatment decisions. Though they may be able to get beyond some of the limits of culture-based approaches, molecular methods and non-culture-based methods like mass spectrometry nevertheless have some of their own [3].

As a result of these constraints, new diagnostic procedures are required. In this thesis, we emphasize the advantages of microfluidic-based diagnostic devices as a solution to these issues.

Antibodies have shown to be widely utilized ligands with several achievements in identifying pathogenic cells in commercial and research systems. Aptamers, on the other hand, stand out as the preferred ligands for the development of sensitive and reliable diagnostic tests when compared to antibodies and other research molecules due to benefits such as small size, low cost, and simplicity of chemical modification. Aptamers can be used as research compounds in a variety of ways. Despite these potential, comprehensive comparisons of aptamer-based biosensors have largely focused on protein analytes.

The aim of this thesis is to eliminate this limitation by systematically comparing the performance of antibodies and aptamer ligands on glass surfaces in the detection of *Escherichia coli, Staphylococcus aureus* and *Acinetobacter baumannii*, which are the most common pathogens associated with sepsis in Turkey.

The biosensor development potential of these ligands was examined through a series of systematic tests, and insights into their distinct performances are presented. The comparison study comprised 12 ligands, and their performances were extensively evaluated. Anti-*E. coli* antibody (HRP) was used for *E. coli*, mouse monoclonal to *S. aureus* for *S. aureus*, and rabbit anti-*A. Baumannii* Elongation Factor Tu Polyclonal Antibody (tuf1) for

A. baumannii. We performed an additional investigation to corroborate the performance of the top-performing ligands by determining the linearity, analytical sensitivity, and repeatability of the results obtained.

The results of this research shed light on the efficiency of antibody and aptamer ligands, indicating that while antibody ligands continue to outperform aptamers in overall performance, aptamers remain a significant promise as an analytical tool. The findings shed light on the selection and use of ligands for biosensor development, specifically in the context of pathogen detection and sepsis diagnosis. Thus, we exhibited a biosensor format that will be used in microfluidic applications by offering significant insights.

Overall, this study adds to our understanding of the comparative performance of antibody and aptamer-based biosensors, highlighting the benefits of aptamers and focusing on their potential as a promising alternative for the development of advanced diagnostic tools in a variety of biomedical applications. Through comparative investigations, we demonstrated the great potential of antibody and aptamer-based biosensors on glass surfaces in the detection of sepsis illness in microfluidic systems.

2. LITERATURE RESEARCH

Sepsis is basically defined as a life-threatening disease with organ failure caused by an unbalanced immunological response to infection. Sepsis is also characterized by physiological, pathological and metabolic problems caused by infection. Sepsis disease causes tissue and organ damage to the body's defense system against infection and therefore has a fatal effect. This disease poses a significant public health problem and is estimated to have caused over $20 billion in hospital expenditures in the United States in 2011 [4]. According to the report of the World Health Organization, it is estimated that sepsis disease affects more than 30 million people worldwide every year [5].

Although sepsis is recognized worldwide as a deadly disease with serious implications, the true prevalence of sepsis disease is uncertain. The main reason for the uncertainty of the prevalence of sepsis is the presence of conditions such as underdiagnosis, misdiagnosis, and differences in reporting standards in different regions and different health systems. Although its prevalence is unknown, the public health effects of sepsis, its high morbidity and mortality rate, have made sepsis a serious problem worldwide. Therefore, monitoring, diagnosis and management options need to be developed to combat the often-fatal disease sepsis [6]. Infections that cause sepsis are fatal, as they cause inflammation and prolonged stay in the intensive care unit (ICU). Sepsis is characterized by a dysregulated immune response to infection. And it actually causes organ damage with the intense inflammation that occurs as a result of the disease. Sepsis disease turns into severe sepsis or septic shock if not treated quickly and appropriately. The worsening of sepsis or its transformation into septic shock also makes it difficult for patients to recover from this disease. So much so that the disease causes an increased risk of organ failure and death at this point [7]. Indeed, what distinguishes sepsis from a simple infection is the presence of an abnormal and dysregulated host response to infection that causes organ failure. Therefore, the first precaution to be taken is to prevent the delay in the diagnosis of the disease and to initiate appropriate treatment before it turns into severe sepsis and septic shock.

If sepsis is not diagnosed and treated quickly, this contagious disease causes many deaths. As a result, sepsis requires rapid detection, urgency, and high priority, both to ensure the patient's survival and not to allow the disease to affect other people [4].

Bacteria, viruses, and fungi are accepted as the most common pathogens causing sepsis. *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* are the most detected bacteria in sepsis patients. *S. aureus* is a gram-positive bacterium and is known to cause a variety of diseases, from skin and soft tissue infections to serious bloodstream infections. The resistance of *Staphylococcus aureus* (MRSA) to some methicillin-containing drugs is a particular concern regarding the treatment of this disease. *E. coli* is a gram-negative bacterium that causes urinary tract infections and causes the development of gastrointestinal diseases. Another pathogen that causes sepsis is *A. baumannii*, a gram-negative bacterium. *A. baumannii* is a serious nosocomial (hospital-acquired) pathogen. This poses a great risk as it causes the disease to spread rapidly in hospitals. *A. baumannii* is mostly seen in healthcare settings and can cause bloodstream infections in people with severe conditions. *E. coli*, *S. aureus*, and *A. baumannii* are often seen as common pathogens in bloodstream infections. Of course, the frequency of these pathogens varies according to geographies and health settings. *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),* and *Acinetobacter baumannii (A. baumannii)* in Turkey and in many regions, it is the most common microorganism causing bloodstream infections (BSI) [8]. In addition, local epidemiology, patient demographics, and drug resistance tendencies are also parameters that affect the incidence of bacteria causing bloodstream infections [9].

"Early onset neonatal sepsis" is an infection that begins within the first 72 hours of life. According to studies, it has been determined that the main bacterium responsible for early-onset neonatal sepsis, especially in the neonatal period, is *E. coli. E. coli*, a gram-negative bacterium, can cause disease when it enters the circulation or crosses natural barriers. Early-onset neonatal sepsis (EOS) becomes more worrisome if *E. coli* bacteria produce extended-spectrum beta-lactamase (ESBL) enzymes. The reason why neonatal sepsis is so deadly is that the immune system is not settled during this period and the baby's body will not be able to handle the severe infection. Especially, premature babies are more likely to get sepsis due to weak immune systems and susceptibility to infections [10]. EOS infections are usually passed through the placenta during pregnancy or from mother to child at birth. Unfortunately, neonatal sepsis remains one of the most important causes of neonatal morbidity and death, despite the precautions and prenatal checkups [11].

S. aureus bacteria is the second most common bacterial group that causes sepsis. Some of the important reasons why *S.* aureus bacteria are dangerous are its biofilm forming

capacity and high affinity for foreign substances. Because of these properties, *S. aureus* greatly increases the risk of infection in people who have medical devices such as catheters, prosthetic joints, heart valves, and pacemakers. When *S. aureus* colonizes these foreign substances, it forms a protective biofilm against the immune system and antimicrobial treatments. This biofilm formation greatly complicates the treatment of sepsis caused by *S. aureus*. Unfortunately, sepsis from *S. aureus* is highly fatal and requires immediate medical attention. When *S. aureus* bacteria enter the circulation, it causes more serious problems than systemic infections such as organ failure, severe sepsis, and septic shock. In general, the biofilm-forming propensity of *S. aureus* and its interaction with foreign bodies contribute to the resulting lethal outcome. Therefore, sepsis caused by *S. aureus* results in high mortality [12]. Therefore, it is critical to prioritize prevention, early detection, and appropriate care to combat *S. aureus* infections and improve patient outcomes [13].

A. baumannii is a nosocomial pathogen, which means that it is often contagious in healthcare environments. This bacterium causes infections in a variety of anatomical areas, including the skin, blood vessels, lungs, and wounds. It is especially common in intensive care units (ICUs), which house vulnerable patients with impaired immune systems and frequently perform invasive medical operations.

Infections caused by *A. baumannii* can appear as hospital-acquired pneumonia, bloodstream infections, surgical site infections, urinary tract infections, and wound infections. Because *A. baumannii* has the capacity to acquire resistance to numerous medicines, including routinely used antibiotics, these infections constitute a substantial issue [14]. The case fatality rate for *A. baumannii*-caused bloodstream infections is between 50% and 60%, according to data. This shows that a significant percentage of patients who get *A. baumannii* bloodstream infections die [15].

Despite its worldwide importance, sepsis is still largely obscure among the general population, demonstrating a low degree of public awareness. Sepsis is a potentially fatal disorder caused by the body's response to infection, and it can result in organ failure, long-term disability, and even death if not diagnosed and treated swiftly [16]. The paucity of public understanding regarding sepsis is troubling, given the significant impact it has on healthcare systems and patient outcomes.

One of the main reasons sepsis is difficult is the wide range of symptoms. In fact, it is difficult for even healthcare professionals to diagnose. Given these challenges, it is necessary to develop a public definition of sepsis in order to raise awareness. In addition, the development of clinical guidelines and diagnostic technologies for early detection and accurate diagnosis is critical. Prompt administration of the right antibiotic therapy in the treatment of sepsis is critical to ensure the survival of patients. Delaying initiation of the correct antibiotic therapy for the treatment of sepsis increases mortality from septic disease. The importance of early diagnosis of sepsis has been demonstrated by studies, with the death rate increasing every hour in patients after the first six-hour delay following the initiation of appropriate antibiotic therapy. These findings demonstrate the importance of time for sepsis detection and the need for rapid detection, diagnosis, and initiation of appropriate medication [17]. Initiating the wrong antibiotic therapy significantly reduces the patient's chance of recovery. Therefore, choosing the right antimicrobial drug for the treatment of sepsis disease is critical. Indeed, studies have shown that starting the wrong antibiotic therapy increases the risk of death in septic shock patients by up to five times. Delay of appropriate treatment causes septic shock. Septic shock is the most severe form of sepsis, with a systemic inflammatory response that causes organ failure and dangerously low blood pressure. Treatment of the underlying infection and management of the course of septic shock need prompt delivery of suitable antimicrobial medication. Beginning the incorrect antimicrobial medicine might have a negative impact on patient outcomes. Improper treatment leads to the inability of pathogens to prevent the disease, causing the infection to continue and worsen. Inadequate treatment leads to persistent inflammation, antibiotic resistance, and even permanent organ damage after sepsis. Appropriate antibiotic therapy is critical in the treatment of sepsis to prevent the patient from dying, recovering, or even spreading the disease to others [18].

Various procedures and approaches are used to detect sepsis clinically. Different methods used to detect sepsis:

Blood culture tests are a standard diagnostic method for identifying infections in the bloodstream. This method involves taking a blood sample from a patient suspected of having an infection and incubating it in a specific culture medium to allow growth of the bacteria or fungus present. Blood cultures are generally incubated for up to 5 days to allow for bacterial

or fungal growth and detection of bacteria or fungi. This long incubation period is a very critical period for the patient's beard. Blood culture tests are vital to know their limits and take precautions, even if they are still an important technique for identifying circulatory infections. So that some bacteria can grow slowly or be present in low concentrations in circulation, which can cause longer incubation times. False negative results or delayed diagnosis in blood culture tests are one of the biggest problems. Also, if any antibiotic treatment is given to the patient before the blood culture test, it may limit bacterial growth or cause the culture results to be incorrect [19]. Blood cultures are a method in sepsis that often has limitations in identifying bacteria. Despite the large number of blood samples taken, blood cultures can only detect pathogens in 40 to 60 percent of patients with sepsis. This rate provides a very low reliability for a fatal disease [20].

Nucleic acid-based methods are an alternative method for bacterial identification for the detection of sepsis disease. Although conventional PCR methods are used for the detection of many diseases today, the risk of contamination is high when working with amplifiers. For this reason, when working with PCR methods, it is necessary to reduce the risk of contamination such as separate working environments, use of special equipment, and repeated laboratory applications by using appropriate controls and to obtain accurate results.

Another method is real-time PCR, which is more reliable, more efficient and has less risk of contamination than conventional PCR methods. Real-time PCR provides real-time monitoring of the amplification process, enabling the identification and determination of target nucleic acids. This approach has advantages such as being faster, more sensitive and identifying multiple pathogens in a single reaction.

Despite all these advantages, real-time PCR procedures require special equipment and reagents. It is therefore more expensive than conventional PCR methods. In addition, many real-time PCR equipment have efficiency constraints that affect the scalability and speed of the test in high-volume cases. The use of nucleic acid-based methods such as real-time PCR requires special tools and experienced workforce, so the application of the method is limited. Adequate training and quality assurance methods are required to provide consistent and trustworthy results. In summary, as compared to traditional PCR methods, nucleic acid-based approaches, particularly real-time PCR, provide advantages in terms of

reliability, efficiency, and reduced danger of contamination. They may, however, be more expensive, need specialized equipment, and necessitate skilled workers. These variables must be considered while choosing on the best nucleic acid-based strategy for sepsis diagnosis, as well as when ensuring suitable implementation and quality control mechanisms are in place [21].

Biochemical studies and immunological assays are two methods routinely utilized to diagnose bacterial infections in sepsis. Each strategy has advantages and disadvantages. Biochemical studies involve the measurement of particular biochemical markers or metabolic products linked to bacterial infection. C-reactive protein (CRP), procalcitonin (PCT), and cytokines are examples of these indicators. Although biochemical tests are widely used, the results of these tests cannot always be guaranteed accuracy and do not show specificity for all results. The fact that it takes a long time to get the results of biochemical tests may delay the application of the correct treatment.

Another way for detecting sepsis is to employ enzyme-linked immunosorbent assay (ELISA) assays. ELISA tests are based on the immunological testing premise, which includes identifying of specific antibodies or antigens linked with bacterial infections. ELISA tests have a high specificity and sensitivity, making them a somewhat excellent means of identifying bacterial infections. The range of bacteria that can be detected by a particular ELISA test, on the other hand, is quite limited. To detect all bacterial infections, an analysis using a broad ELISA test panel is necessary. At the same time, using a large ELISA panel is unfortunately expensive, time-consuming, and necessitates extensive laboratory knowledge [22].

Another method, microarray-based diagnostics, uses a microarray containing gene segments created via PCR to detect bacterial infections that cause sepsis. In this method, it enables more than one target gene or DNA sequence to be studied in a single study. To obtain accurate and comprehensive results, the microarray analysis must include the gene segments of various pathogens that cause sepsis. However, it is very difficult and costly to create a comprehensive microscope of all the pathogens that cause sepsis. In short, in order to specificity and precision with this method, the scope, design and correct implementation of microarray is critical. At the same time, in microarray technology, certain microarray chips,

reagents, and equipment are very expensive, so they are more expensive than other diagnostic procedures. Finally, for the processing and interpretation of microscopic data, bioinformatics requires high-quality analysts and specialized software tools, in which case they are the barrier parameters for a fast and cost-effective diagnosis [23].

FISH (fluorescence *in situ* hybridization) is another molecular method used to detect circulatory infections. FISH analyses are based on the use of oligonucleotide probes that are specifically designed to target bacteria or fungal genes. FISH analyses allow pathogens to be detected directly in clinical samples without requiring any amplification before the test. FISH analyses basically hybridize fluorescent labelled probes into the corresponding target series within the pathogen genome. This enables the bacteria to be detected and visualized directly under a fluorescent microscope. To successfully detect and identify the infections, specialized probes targeting the diseases' genetic sequences must be provided. It might be difficult to build and get adequate probes for a wide range of diseases. Furthermore, FISH is largely a qualitative technology that provides visual confirmation of the existence of microorganisms but does not provide quantitative information such as bacterial load or accurate species identification. The interpretation of FISH data necessitates microscopy competence as well as understanding of the pathogens being studied. The usefulness of FISH is limited due to the vast array of bacteria that may cause bloodstream infections. It may be more appropriate in circumstances when specific diseases are suspected or when quick imaging of germs is required. Additional diagnostic procedures, such as culture-based techniques or molecular tests targeting specific genetic markers, may be necessary for a full and reliable diagnosis in settings with a larger variety of possible infections.

In essence, FISH is a rapid and easy molecular approach for seeing bacteria or fungus in clinical samples. However, the availability of specialized probes and the diversity of bacteria causing bloodstream infections restrict its diagnostic breadth. To overcome these limits and provide a fuller assessment of the infecting microorganisms, complementary diagnostic techniques may be required [24].

Bacteriophage-based pathogen detection tests are a form of phenotypic test that uses viruses that infect and proliferate within bacterial cells. These assays have various benefits, including the ability to directly visualize labeled phages, identify offspring phages formed

during infection, and use of reporter phages that convey traceable signals inside their bacterial hosts. Bacteriophage-based tests may identify diseases quickly and specifically by targeting certain bacterial strains. Bacteriophage replication within their host cells causes signal amplification, enabling for sensitive detection. Because they do not require the extensive incubation periods required for bacterial growth, these tests may offer shorter turnaround times than standard culture-based procedures. However, investigations on the technical issues and general application of bacteriophage-based pathogen identification assays for sepsis diagnosis are still few. There is a scarcity of thorough data on the performance, sensitivity, and specificity of these tests, particularly in the setting of sepsis. More research and validation studies are needed to determine their efficacy in clinical settings [25].

Proteomic methods, such as matrix-assisted laser desorption-ionization flow mass spectrometry (MALDI-TOF MS), have demonstrated efficacy in detecting bacteria or fungus based on proteomic patterns. This method includes studying the distinct protein patterns of microorganisms to distinguish between various species or strains. However, MALDI-TOF MS and other proteomic approaches need a substantial number of microbial cells for analysis. When detecting sepsis, where the bacterial load in the circulation is low, extra procedures are frequently necessary to get a valid sample for examination. This usually entails blood culture, which permits bacteria to grow and multiply before proteomic analysis can be undertaken. The requirement for previous culture and following steps in the proteomic analysis process might cause the entire diagnosis procedure for sepsis to be prolonged. This delay in collecting data may not be optimal for the timely management of septic patients when quick identification and therapy are critical. In conclusion, proteomic methods such as MALDI-TOF MS have shown useful in bacterial identification and characterization based on protein patterns. However, the method they use to detect sepsis has limitations due to the need for prior culture and additional processing, which may prolong the diagnostic procedure. Septic patients need methods that can provide faster and more direct detection. In this way, the treatment and optimal care of patients will be improved [26].

Early detection of sepsis, correct diagnosis, and appropriate treatment are critical to minimizing the severity of the disease. Traditional sepsis diagnostic procedures are mostly

performed on blood culture reports followed by molecular diagnostic tools. However, many of these approaches are costly, lengthy, and require skilled laboratory staff. It also has disadvantages such as insufficient sensitivity and specificity.

As a result, there is a need to develop different sepsis diagnosis systems. The use of highly sensitive biosensors seems to be a promising technique for rapid diagnosis and detection of sepsis disease. In recent years, various biosensors have been developed for the detection of sepsis using various biomarkers that play an important role in the pathophysiology of sepsis. These biosensors have proven to be successful and there is promise in the development of new biosensors.

Biosensors have many outstanding features for their use in the detection of sepsis disease. The identification of biomarkers in real time allows for the early and definitive diagnosis of sepsis disease. Biosensors are very advantageous because they can detect even low concentrations of biomarkers. Thanks to these characteristics, more specific results are obtained. In addition, biosensors can be made portable, user-friendly, and suitable for point-of-care applications, allowing fast and simple testing in various clinical situations. The development of biosensors for the detection of sepsis disease facilitates accessibility, as well as improving diagnostic accuracy and efficiency.

By using the potential of biosensors, it is possible to overcome the limitations of current diagnostic approaches and enable the early diagnosis of sepsis disease. Therefore, biosensors show high promise in terms of early detection of sepsis disease and timely administration of correct antibiotic therapy. biosensors are critical to the improvement of patients. Traditional diagnostic procedures have their limits, and biosensors have the potential to exceed these limits. Biosensors are very advantageous with their high sensitivity, fast detection capability and the potential to be applied as portable. Ongoing research and development in biosensor technology for the detection of sepsis disease demonstrates the high potential of biosensors [27].

In recent decades, the biosensor development industry has made significant progress due to the growing need for the development of microfluidic biosensors with portable potential for point-of-care testing. Microfluidics are based on manipulating and controlling small amounts of liquids within microscale channels and chambers. Microfluidic biosensors

for the diagnosis of diseases have several advantages. It enables fast and accurate patient sample analysis by enabling the integration and automation of complex laboratory processes on a single chip. Biosensors are portable and allow faster diagnosis, treatment, as they eliminate the need for samples and centralized laboratory facilities. At the same time, it eliminates many limitations because it provides real-time and on-site monitoring.

The combination of microfluidic technologies with biosensors enables the identification of specific biomarkers associated with sepsis disease and other diseases. Such biosensors provide the possibility to detect a wide range of analytes, including proteins, nucleic acids, and small compounds, with great sensitivity and specificity. The microscale size of the biosensors reduces the detection time and thus improves the biosensor performance.

Microfluidic biosensors for disease detection testing provide a variety of uses, including hospitals, clinics, and places without hospital or laboratory access. It is frequently used in the rapid diagnosis of infectious diseases, monitoring of chronic disorders and detection of sepsis biomarkers. Microfluidic biosensors have a strong potential in terms of wide usage area due to their ease of use, protection of their mobility and enabling multiple analysis. They have high advantages over traditional diagnostic procedures with their sensitivity, specificity, fast analysis time, low cost, and preservation of mobility [28].

Proteins are often used as target molecules in disease diagnostic tests, usually of microfluidic technologies. Immunoanalysis is preferred due to its simplicity, speed, and ease of use. In order to detect the presence of a specific protein biomarker, these analyses are based on the principle that antibodies specifically capture the target antigen. Lateral flow tests work according to the principle of capillary action, in which the sample passes through microfluidic channels and antibodies, or antigen-antibody complexes fixed to a test strip interact. The outcome often appears as a color shift or a signal line that shows whether the target protein is present or not. Specific illness indicators may be found by using proteins as target molecules, making it easier to diagnose and keep track of a variety of disorders [29].

To enable immunological testing, biosensors, and microfluidic devices for the detection of bacterial infections, antibodies produced against bacterial proteins are frequently immobilized on surfaces. These antibodies enable the selective capture and

identification of the target bacterial antigens by binding to them precisely. One commercially available system that makes use of immunological testing for point-of-care diagnostics is Alere Triage. This system was made for rapid and accurate identification of various analytes such as bacterial proteins in clinical samples. Microfluidic technology was used in the system and antibodies against certain bacterial antigens were immobilized on the surface of the device. Thus, when the patient sample is inserted, the target bacterial proteins in the sample bind to the immobilized antibodies and convert them into a measurable signal such as color change or fluorescence that indicates the presence of the pathogen. Microfluidic systems allow sensitive and specific detection of bacterial infections based on the potentials of antibodies, ligands, and immunological responses. These systems have advantages such as fast results, simplicity, and portable application possibility. Microfluidic systems are critical to detect bacterial infections early and accurately, enabling faster determination of treatment modality and analysis of patient outcomes [30].

Combining microfluidic polymeric chips with antibody-labeled gold particles has emerged as an alternative method to typical lateral flow investigations for the detection of proteins. This approach increases the sensitivity and specificity of the capture of bacterial proteins. In this method, microfluidic polymeric chips serve as a platform for the assembly and detection of bacterial proteins. There are special capture antibodies on the surface of the chip that are immobilized and can selectively bind to target proteins. Colloidal gold particles were labeled with antibodies targeting bacterial proteins of interest. When target bacterial proteins are placed on the chip, they bind to capture antibodies on the surface of the chip. Gold particles are then injected and bound to trapped bacterial proteins that are labeled to detect antibodies. This binding event is visualized as a signal such as a color change or a fluorescent signal indicating the presence of target proteins. This method has several advantages over typical lateral flow tests, such as higher sensitivity and specificity.

The use of microfluidic polymeric chips allows precise control of sample flow and reagent mixing, and detection by providing more efficient aggregation of bacterial protein. The tagged gold particles provide a strong and visible signal, enabling data to be understood. With this approach, it has shown 99% success for protein identification and successful results in the case of myocardial infarction. Continued advances in microfluidic technology and antibody labeling techniques promise to improve the sensitivity, specificity, and overall

performance of these microfluidic polymeric chip-based protein detection systems [31]. Indeed, the attachment of antibodies to PDMS (polydimethylsiloxane) surfaces has permitted the construction of microfluidic devices for analyzing different blood components. Numerous studies have shown that this method has pioneered the way for protein binding analysis in microfluidic devices. It should be noted, however, that microfluidic methods utilized for protein determination may have a time disadvantage. For effective binding and detection, they often require unbound proteins in the sample. If the protein of interest is present in complex matrices, such as blood, and is accompanied by viral or bacterial proteins, sample lysis and processing may be required before analysis to isolate and concentrate the target protein. Nucleic acids, like proteins, have found use in microfluidic lab-on-a-chip technology. These devices enable the examination of nucleic acids in a tiny format, such as DNA or RNA. Sample preparation, amplification (e.g., polymerase chain reaction), and detection (e.g., fluorescence-based assays) may all be performed on microfluidic devices. This allows for rapid and sensitive identification of specific genes or nucleic acid targets of infections. It provides accurate and reliable diagnostics by combining nucleic acid analysis and protein analysis in microfluidic on-chip laboratory devices. Thus, it expands the field of application by enabling the detection of protein and nucleic acid-based biomarkers and thus increases the diagnostic potential of microfluidic technology. Advances in microfluidic on-chip laboratory technologies, such as sample processing and the adaptation of multiple detection methods, show great potential for the development of more efficient diagnostic platforms that can analyze both proteins and nucleic acids in a single device [32].

Nucleic acid analysis provides accurate results but may not be ideal for direct application in microfluidic systems due to the extra steps required, such as DNA replication and cell lysis. These procedures are more complicated and require more time. This reduces the specificity and speed of diagnosis for each patient. To overcome this limitation, the researchers focused on the direct detection of bacterial cells in microfluidic biosensors in order to reduce the sampling and analysis time. It is intended that these biosensors will be designed to target surface components such as proteins, glycoproteins, lipopolysaccharides, and peptidoglycans for the identification of the entire bacterial cell. Unlike bacterial antibodies, which typically target specific molecules, various polyclonal antibodies are created for wider recognition. The goal is to create biosensors that can detect the presence

of all bacterial cells and allow for a more thorough and rapid investigation of infectious agents.

These biosensors simplify the diagnostic procedure by focusing on the detection of bacterial cells instead of other molecular targets. Thus, they have the potential to provide faster findings. However, further analysis is needed to identify the exact surface molecules in order to improve the specificity, sensitivity and selectivity of these biosensors and to use them as useful targets in antibody recognition.

The use of microfluidics in the creation of biosensors capable of detecting entire bacterial cells is a promising method. This method allows for the rapid and accurate detection of pathogens without the need for sampling and amplification processes, which is an important step [33]. As a result, monoclonal antibodies can be produced against specific target proteins [34]. Specially designed microfluidic chips have been designed for the detection of *Vibrio cholerae* and *E. coli* [35]. The use of surface antibodies in microfluidic technology increases the belief in the identification of bacteria and the creation of electrochemical biosensors. Microfluidic devices have many advantages, including precise fluid flow control, reduced sample volume, and the integration of various functional parts on a single chip. Surface antibodies bind to the microfluidic chip surface to identify bacteria, allowing for the capture and recognition of microorganisms specifically targeted. These antibodies can be designed to target cell surface components of various bacteria, including proteins, glycoproteins, lipopolysaccharides, and peptidoglycans. Furthermore, electrochemical biosensors provide measurable signals using the electrical properties or interactions of captured microorganisms.

Electrochemical biosensors embedded in microfluidics provide great sensitivity and the potential to downsize portable devices for disease detection applications. They have showed promise in a range of clinical diagnostic applications, including identifying food safety. The researchers created a high-potential approach for quick and accurate detection of bacterial infections by combining the specificity of surface antibodies with the sensitivity and mobility of microfluidic electrochemical biosensors. These methods have resulted in the creation of potent and effective new diagnostic instruments that can aid in the early diagnosis and treatment of bacterial diseases [36].

The development of microfluidic biosensors for the detection of *E. coli* and *S. aureus* has introduced several methodologies and hurdles. One method for detecting *E. coli* includes a sandwich test with *E. coli*-specific surface antibodies by surface activation of chitosan followed by formation of biotin-streptavidin complexes. This technique attempted to collect and identify *E. coli* pathogens through specific antibody-antigen interactions. However, the system's intricacy made working at the bedside challenging. Antibodies specific to protein A were adapted onto microbeads and used in a lab-on-a-chip (LOC) microfluidic approach to detect *S. aureus*. This method detected *S. aureus* at concentrations as low as one colony-forming unit (CFU) per mL. The use of the tetrathiafulvalene system for signal amplification, which might increase detection sensitivity, was unsuitable for bedside analysis in this method. Research is still being done to develop microfluidic biosensors that can be used at the bedside, are affordable, can evaluate whole bacterial cells, and can simultaneously study a variety of bacterial species. These biosensors are made to detect bacteria precisely and rapidly, enabling swift diagnosis and treatment choices [37].

Immunosensors and magnetic sensors are very important for the detection of sepsis disease. Immunosensors based on antibody-antigen binding have found widespread use in disease detection and food safety. Antibodies are often used as ligands in immunosensors due to their high affinity and reliability. When antibodies are used as ligands, they have advantages such as sensitivity, reproducibility, and ease of use. In fact, immune sensors detect some of the biomarkers associated with sepsis, allowing early and reliable detection of the disease. Magnetic sensors, on the other hand, collect and detect target antigens by using customized magnetic nanoparticles and antibodies together. This method not only provides fast and effective detection, but also enables its use in portable devices and point-of-care testing. Combining magnetic sensors with immune sensors creates a powerful technique for sepsis diagnosis with improved sensitivity, accuracy, and speed. Continuing work in this industry is improving the development and application of these technologies for sepsis and other infectious diseases [38].

Aptamer-based aptasensors, on the other hand, have great potential in a variety of applications, including sepsis detection. Because aptamers have several advantages over typical antibodies. Aptamers are highly stable, non-immunogenic, and chemically exchangeable. In addition, aptamers are smaller in size than antibodies, so they are

advantageous in many application areas. The different properties of aptamers enable them to detect and bind to specific target molecules in the same way as monoclonal antibodies. They can be designed or selected to have high affinity and specificity for their targets, making them advantageous in biosensing applications. Aptamer-based aptasensors can detect sepsis-related biomarkers in the setting of sepsis diagnosis with excellent sensitivity and accuracy [39]. Recent research has shown that you have a high potential to create highly sensitive detection platforms for the diagnosis of diseases in aptamers and other nanomaterials. These new technologies combine the superior properties of nanomaterials with the unique characteristics of aptamers, which have high affinity and specificity. Researchers have managed to develop systems that enable early and accurate diagnosis of a large number of diseases by combining aptamers with nanomaterials. Further work in this sector is expected to make a significant contribution on a larger scale to the identification and prevention of diseases [40].

Aptamer-based sensors evaluate samples based on the detection of the conformational changes experienced by aptamer molecules when they bind to specific target analytes. The signals produced by the aptamer-based sensors are detected and measured using these conformational changes. Typically, aptamers are made in the lab using a process called systematic evolution of ligands through exponential enrichment (SELEX). Thus, during SELEX, aptamers with certain properties can be selected from a large library of oligonucleotides with diverse sequences and complex structures. Through several rounds of selection, aptamers with strong affinity and specificity to the target site are created. When these aptamers bind to their targets, they undergo certain conformational changes, and these changes are translated into detectable signals in aptamer-based sensor devices. The flexible nature of aptamers and their capacity to undergo conformational changes when bound to a target make aptamers useful tools for detecting and detecting applications. The SELEX method enables the creation of aptamer-based sensors useful for biological research, diagnostics and beyond by producing aptamers with excellent specificity and sensitivity to target points [41].

Several approaches for identifying harmful germs with great sensitivity and selectivity have been developed. However, the instability and limited shelf life of antibodies, as well as their high cost, sensitivity to cross-reactivity, unpredictability in production, and

intricate manufacturing methods, offer significant difficulties. Aptasensors, which offer various benefits over antibodies, have emerged as a viable alternative. Aptasensors that are unique to a bacteria can interact with the cell surface directly rather than through nucleic acids. Because of this, they are particularly helpful for identifying whole-cell bacteria without the need for extensive sample preparation methods [42]. Recent research has revealed an increasing interest in biosensors and aptasensors for illness detection. While biosensors have garnered greater attention in the literature and have a bigger body of supporting research for bacterial detection, aptasensors are gaining popularity. However, comparative studies directly evaluating the performance of biosensors and aptasensors for the identification of the same bacterial species using identical detection techniques are lacking. [43]

Nonetheless, certain studies comparing individual proteins have shown that aptamers have intriguing promise. It is suggested that more extensive research be undertaken to determine the efficacy of both biosensors and aptasensors before their deployment in real-world applications and commercialization [44].

We proved the tremendous potential of antibodies and aptamers in the diagnosis of sepsis in this work. We discovered that microfluidic system applications have a high diagnostic potential through our research on glass surfaces.

3. MATERIALS & METHODS

3.1 Materials

3.1.1 Chemicals

The list of chemicals used during the study has been provided as an appendix (APX. Table.1).

3.1.2 Laboratory equipment

The list of laboratory equipment used during the study has been provided as an appendix (APX. Table.2).

3.1.3 Ligands

A detailed literature analysis was undertaken to find the ligands with the most promise for the study, and it was determined to work with the ligands mentioned in the table below. Table 1 contains the entire list of ligands used for the investigation.

Table 1. Complete List of Ligands Used in the Study

Bacteria	Aptamer/Antibody
E. coli	Anti-*E. coli* antibody - Abcam (ab25823)
	Rabbit polyclonal antibodies- Serotec (4329-4906)
	Anti-*E. coli* antibodies (HRP) - Abcam (ab68450)
	Anti- *E. coli* LPS antibodies – Abcam (ab35654)
	Aptamer
S. aureus	Mouse monoclonal [704] to *S. Aureus*- Abcam (ab37644)
	Anti-*S. aureus* Enterotoxins A + B + TSST-1 antibody- Abcam (ab190337)
	Anti-*S. aureus* antibody- Abcam (ab20920)
	Mouse monoclonal [702] to *S. aureus*- Abcam (ab20002)
	Aptamer
A. baumannii	Rabbit anti - *A. baumannii* elongation factor Tu polyclonal Antibody (tuf1) - MYBIOSOURCE (MBS1491803)
	Rabbit anti - *A. baumannii* elongation factor Tu polyclonal Antibody (tuf1), Biotin conjugated - MYBIOSOURCE (MBS1492257)

After determining the suitable ligands for use, the ligands listed in the table were sourced from various companies.

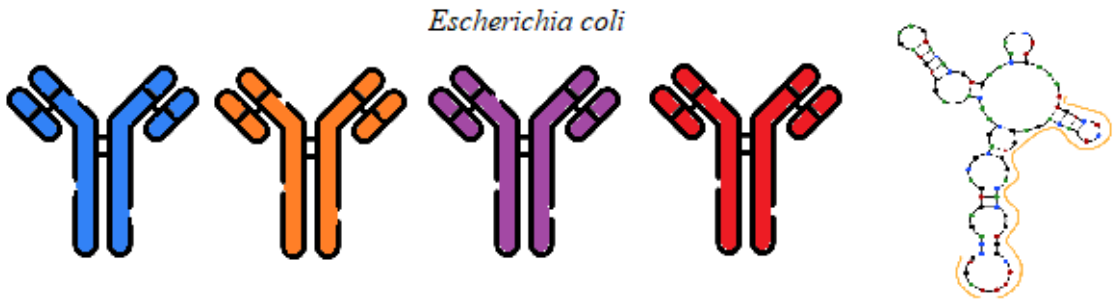

Figure 1. Ligands Selected for *E. coli*

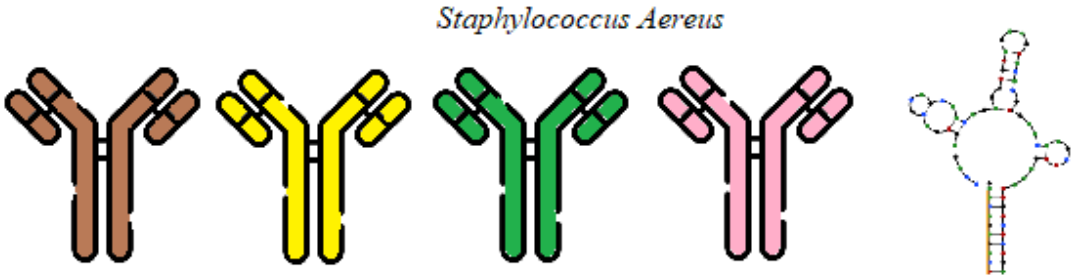

Figure 2. Ligands Selected for *S. aureus*

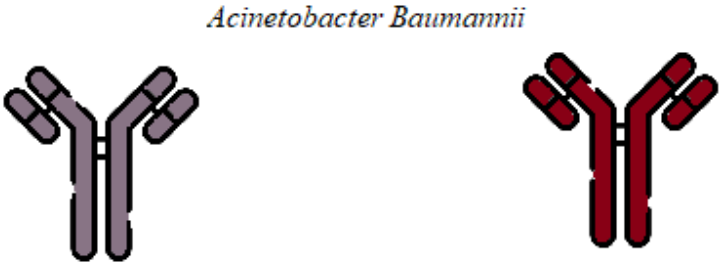

Figure 3. Ligands Selected for *A. baumannii*

3.2 Methods

3.2.1 Glass surface modification and aptamer/antibody immobilization

To immobilize the aptamers [45] on glass surfaces, the following steps performed:

The glass surfaces were transformed to diol silica surfaces by treating them at room temperature for 10 minutes with glycidoxypropyl-trimethoxysilane, followed by 4 hours of heating at 90°C. Following that, the surfaces were rinsed with distilled water, acetone, and ether before being left at 100°C overnight. To activate the surfaces, 0.5 M 1,1'-dry acetonitrile was applied and kept at room temperature for 1 hour before washing with acetonitrile. In a 50 mM sodium phosphate solution, the 3'-amine modified aptamers were produced and dripped onto the activated glass surfaces (Figure 4), where they dried. Phosphate-buffered saline (PBS) was used to remove unbound aptamers from the surfaces. A 0.1 M ethanolamine solution was added to the surfaces, incubated for 2 hours, and then rinsed with PBS to inhibit any residual unbound sites (Figure 5).

These methods permitted aptamer immobilization on glass surfaces, enabling for subsequent investigation and detection of target molecules or microbes.

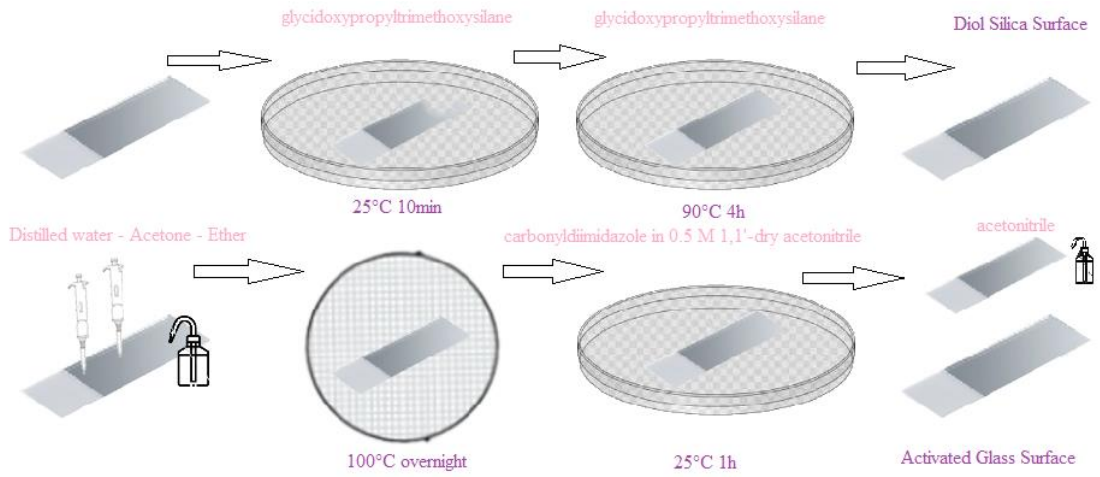

Figure 4. Modification Procedure of Glass Surfaces for Antibody and Aptamer Immobilization

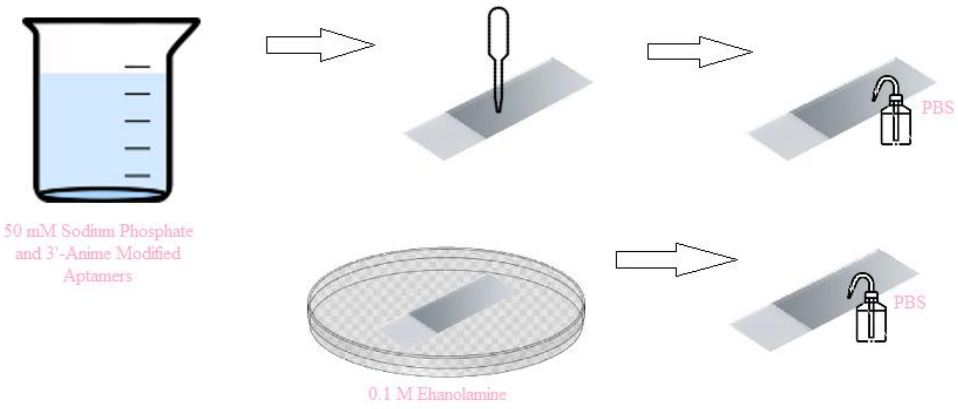

Figure 5. Aptamer Immobilization Protocol

To modify the glass surfaces and immobilize the antibodies [46], the following steps were carried out:

To connect primary amine groups to the glass surfaces, an amino silane solution was used. The glass surfaces were then incubated for 30 minutes in a 3%-aminopropyltriethoxysilane solution to improve the amine functionalization. Separately, glutaraldehyde and the antibody solution were produced. Incubating the glass surfaces in glutaraldehyde solution permits the development of a reactive aldehyde group on the surface. The surfaces were treated in the antibody solution after being incubated in glutaraldehyde, allowing the antibodies to attach to the aldehyde groups on the surface. Washing stages with a 0.05% Tween-20 solution were used to prevent non-specific binding. The proportion of antibody binding was calculated by analyzing the quantity of antibodies in the washing solutions using spectroscopic techniques.

Following these methods, the glass surfaces were changed with primary amine groups and the antibodies were immobilized onto the surfaces, resulting in a surface that can interact with particular antibodies and antigens. The Tween-20 washing phase reduced non-specific binding, resulting in better specificity in the subsequent study (Figure 6).

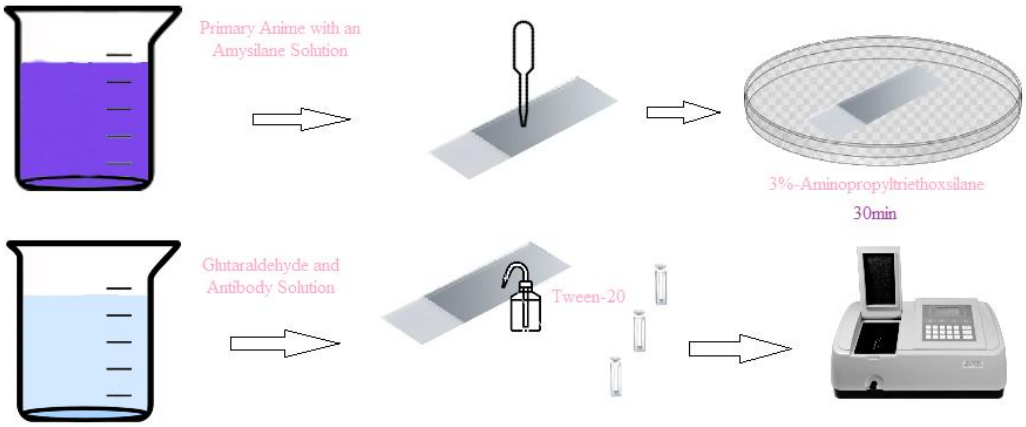

Figure 6. Antibody Immobilization Protocol

3.2.2 Efficiency of aptamer/antibody immobilization

Spectroscopic techniques were used to quantify the quantities of aptamer and antibody in the washing solutions. The purpose was to calculate the aptamer and antibody immobilization binding efficiency.

The success threshold for aptamer immobilization was established at 80% efficiency. To obtain the necessary immobilization efficiency, the incubation periods were adjusted. The binding efficiency was calculated using the initial aptamer concentration ($[C^0_{Apt}]$) and the aptamer concentration in the washing solution ($[C_{Apt}]$). The ratio of the solid phase aptamer concentration to the initial aptamer concentration was determined by the formula $[(C^0_{Apt} - C_{Apt}) / C^0_{Apt}]$, and this value was used to calculate the binding efficiency.

Similarly, the success requirement for antibody immobilization was defined at a minimum of 90% efficiency. Incubation times were adjusted accordingly. The binding efficiency was calculated using the initial antibody concentration ($[C^0_{pro}]$) and the antibody concentration in the washing solution ($[C_{pro}]$). The solid phase antibody concentration was determined based on the initial antibody concentration. The binding efficiency was then calculated using the formula $[(C^0_{pro} - C_{pro}) / C^0_{pro}]$.

The binding efficiency of both aptamer and antibody immobilization was obtained by evaluating the concentrations and applying these calculations, which confirmed the effectiveness of immobilization procedures on surfaces (Figure 7).

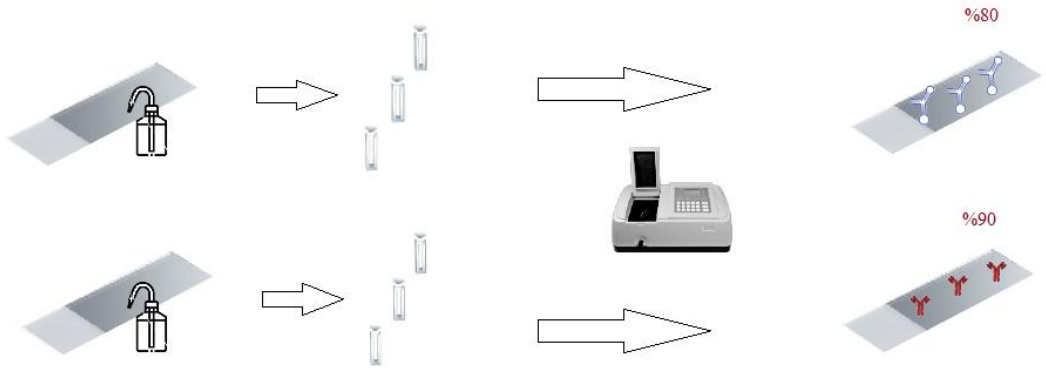

Figure 7. Procedure for Measuring the Immobilization Efficiency of Aptamer and Antibody Ligands

3.2.3 Reproducibility of ligand immobilization

Replica studies were carried out to examine the reproducibility of ligand immobilization using different operators, production batches and time frames. To assess repeatability, the coefficient of variation was determined using the mean and standard deviation data. In total, 12 different ligands were examined, including 4 antibodies and 1 aptamer for *E. coli* (EL-1, EL-2, EL-3, EL-4, EL-5), 4 antibodies and 1 aptamer for *S. aureus* (SL-1, SL-2, SL-3, SL-4, SL-5), and 2 antibodies (AL-1, AL-2) for *A. baumannii*. Each ligand was subjected to 8 replicates (R1, R2, R3, R4, R5, R6, R7, R8), resulting in a total of 96 immobilizations. The goal of these replication tests was to assess the consistency and reproducibility of the ligand immobilization process across batches, operators, and time periods. The variability of the immobilization findings was analyzed by calculating the coefficient of variation, which provided insights into the repeatability of the immobilization technique for each ligand (Figure 8).

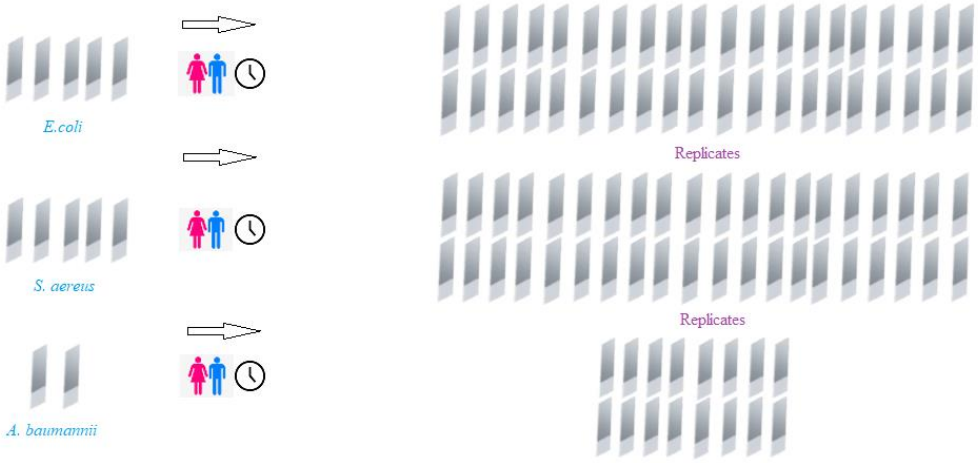

Figure 8. Protocol for Ligand Immobilization Reproducibility

3.2.4 Determination of ligands with high affinity to the target bacterium and not cross- reacting

To evaluate the affinity and non-cross-reactivity of the ligands for the target bacteria, a study was conducted using 4 antibodies and 1 aptamer for *E. coli*, 4 antibodies and 1 aptamer for *S. aureus*, and 2 antibodies for *A. baumannii*. For each of the three microorganisms, solutions containing 10^3 CFU/mL were produced. After that, the produced bacterial solutions were incubated in triplicate on different glass surfaces covered with different antibodies/aptamers. For each bacterium, 36 glass surfaces were constructed, comprising surfaces with non-cross-reacting ligands with high affinity for the particular target microorganisms. The goal was to see how effective these ligands were at capturing microorganisms. Capture efficiency was defined as the ratio of the bacterial concentration at the outlet to the concentration initially introduced at the inlet, minus 1.0. For the calculation of bacteria capture efficiency, the values of $[C^0_{bac}]$ (initial bacterial concentration) and $[C_{bac}]$ (wash bacteria concentration) were used. The solid-phase bacteria concentration was determined by calculating the ratio of $(C^0_{bac} - C_{bac})$ to C^0_{bac}. The bacteria capture efficiency for each ligand was calculated using these formulas. This experimental setting allowed for the evaluation of the ligands' capacity to precisely capture the target bacteria while avoiding cross-reactivity, as well as insights into their effectiveness in collecting bacteria from solution (Figure 9).

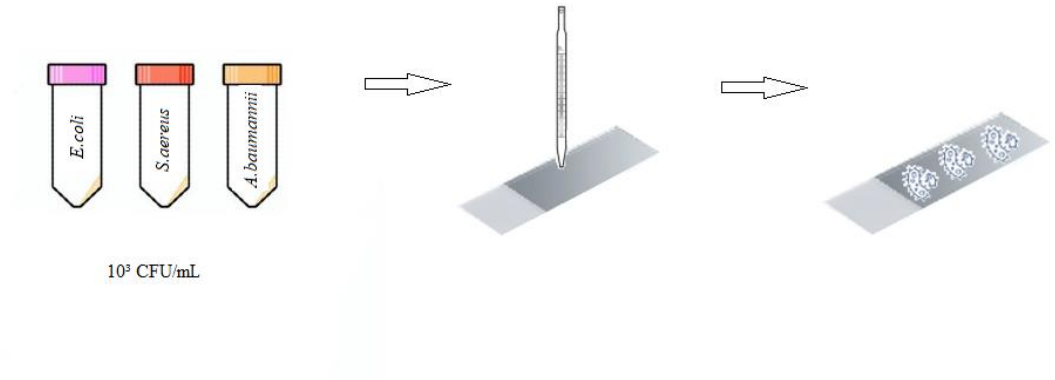

Figure 9. Selection Procedure for Ligands with High-Affinity Binding to the Target Bacteria and Non-Cross-Reactivity

3.2.5 Immobilization of selected ligands and reproducibility

The glass surfaces were initially treated with the amino silane solution to attach the main amine in order to immobilize the specified ligands. The glass surfaces were treated in a 3% aminopropyltriethoxysilane solution for 30 minutes before being incubated in glutaraldehyde and antibody solution. The surfaces were cleaned with Tween-20 at a concentration of 0.05% to avoid non-specific binding.

Different bacterial strains were cultured on distinct agar plates to create bacterial solutions for the studies. *E. coli* bacteria were grown on Muller Hinton Agar, while *S. aureus* and *A. baumannii* bacteria were cultivated on Columbia Agar. The agar plates were then incubated at a temperature of 37°C for a period of 24 hours. Following incubation, a portion of the agar plates containing bacterial colonies was chosen. Bacteria were gathered from a single colony using sterilized loops or other suitable instruments. These colonies were then put into Luria Broth, a liquid growth medium. The bacterial cultures were incubated overnight at 37°C with agitation on an orbital shaker device. The bacterial cultures were then treated to acquire the concentration required for the studies. For 20 minutes, the cultures were centrifuged at 1200 rpm, causing the bacteria to form a pellet at the bottom of the tubes. After carefully removing the supernatant, 20 mL of Phosphate-buffered saline (PBS) was added to resuspend the bacterial pellet. Spectrophotometer measurements at a specified wavelength, commonly 600 nm (OD600), were used to determine the quantity of bacteria in

the solution. 1 mL of PBS was used as a blank, and 1 mL of the bacterial suspension was put in a quartz cuvette with a 10 mm route length. The absorbance of the bacterial sample was determined using a spectrophotometer set to the OD600 program. Bacterial cell counts were calculated using a conversion factor obtained through a calibration process based on the link between optical density and cell concentration. A commonly used conversion factor is OD600 1.0 = 8 x 10^8 cells/mL. By applying this conversion factor to the measured optical density, the concentration of bacteria in the suspension was determined. Finally, the bacterial solution was diluted with phosphate-buffered saline to the concentration required for the tests. To obtain the required concentration, an adequate volume of PBS was added to the bacterial solution and well mixed (Figure 10).

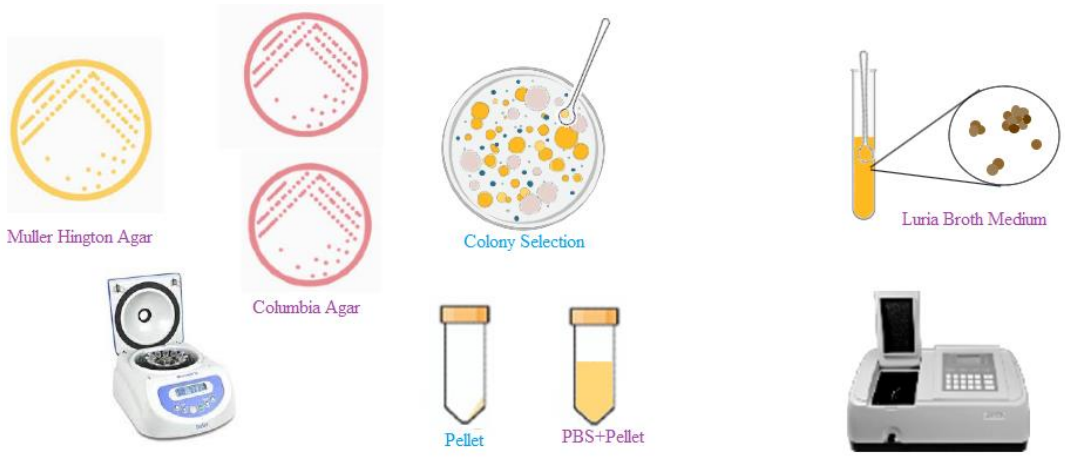

Figure 10. Protocol for Bacterial Culture Establishment and Preparation of Bacterial Solutions

The assay's repeatability was determined by calculating the coefficient of variation using the mean and standard deviation results. This was accomplished through the use of duplicated studies with varied operators, manufacturing batches, and timeframes. Known concentrations of bacteria (100, 200, 400, 600, 800, and 1000 CFU/mL) were prepared for each of the three bacteria. A total of 288 glass substrates were used, including 96 modified glass surfaces with anti-*E. coli* antibodies (HRP) for *E. coli*, 96 surfaces modified with mouse monoclonal antibodies to *S. aureus* for *S. aureus*, and 96 surfaces modified with rabbit anti-*A. baumannii* Elongation factor Tu polyclonal Antibody(tuf1) for *A. baumannii*. Bacteria capture efficiency was calculated as the ratio of the bacterial concentration at the exit to the concentration initially introduced at the entrance, minus 1.0. This was determined by using the values of [C^0_{bac}] and [C_{bac}] to calculate the ratio of solid-phase bacteria

concentration to the initial bacterial concentration using the formula [(C^0_{bac} - C_{bac}) / C^0_{bac}]. Based on this result, the bacteria capture efficiency was estimated. The assay's repeatability was determined by calculating the coefficients of variation using the average and standard deviation values of the bacterial capture efficiency (%). This study revealed information about the assay's consistency and reliability across multiple operators, manufacturing batches, and time periods (Figure 11).

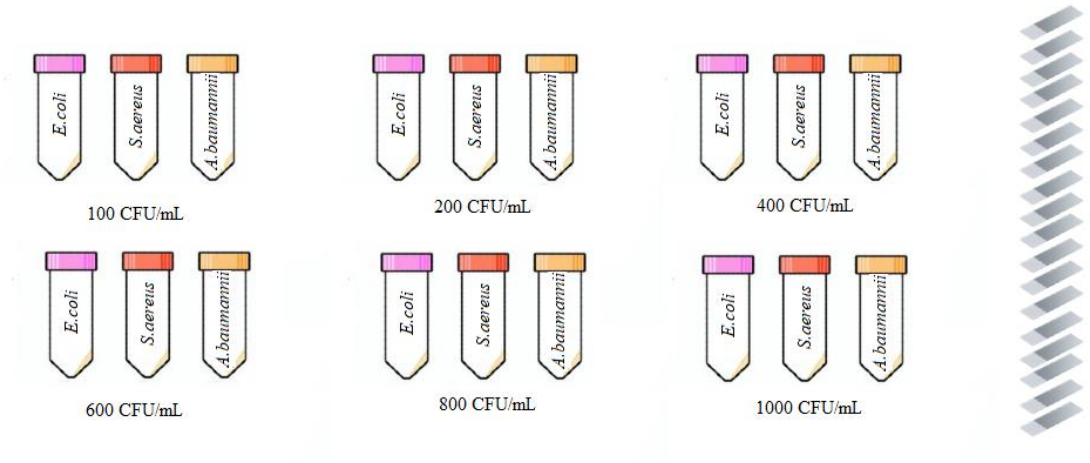

Figure 11. Protocol for Replicability of Ligand Immobilization at Different Concentration Levels

3.2.6 Linear range and analytical sensitivity (limit of detection)

To evaluate the devised system's linear range and analytical sensitivity (limit of detection), 12 solutions were made for each of the three distinct bacteria at variable concentrations ranging from 20 to 1200 CFU/mL. This allowed for the evaluation of the system's performance across a wide range of bacterial concentrations. For *E. coli*, 144 glass surfaces were modified with Anti-*E. coli* antibodies (HRP). Similarly, 144 glass surfaces were modified with monoclonal antibodies specific to *S. aureus* for the detection of this bacterium. For *A. baumannii*, 144 glass surfaces were modified with Rabbit anti-*A. baumannii* elongation factor Tu polyclonal Antibody (tuf1). In total, 432 glass surfaces were used in the study.

The term "linear range" describes the range of bacterial concentrations across which the system can produce outcomes that are inversely proportional to the bacterial concentration. It was possible to calculate the linear range of the system by examining the

responses received from the various bacterial concentrations. The analytical sensitivity, commonly referred to as the limit of detection, shows the smallest number of bacteria that the system is capable of accurately detecting. The system's limit of detection might be established by analyzing the responses produced at low bacterial concentrations. Insights into the system's capacity to precisely measure bacterial quantities within a certain range and identify low concentrations with high sensitivity were gathered through the linear range and analytical sensitivity investigation (Figure 12).

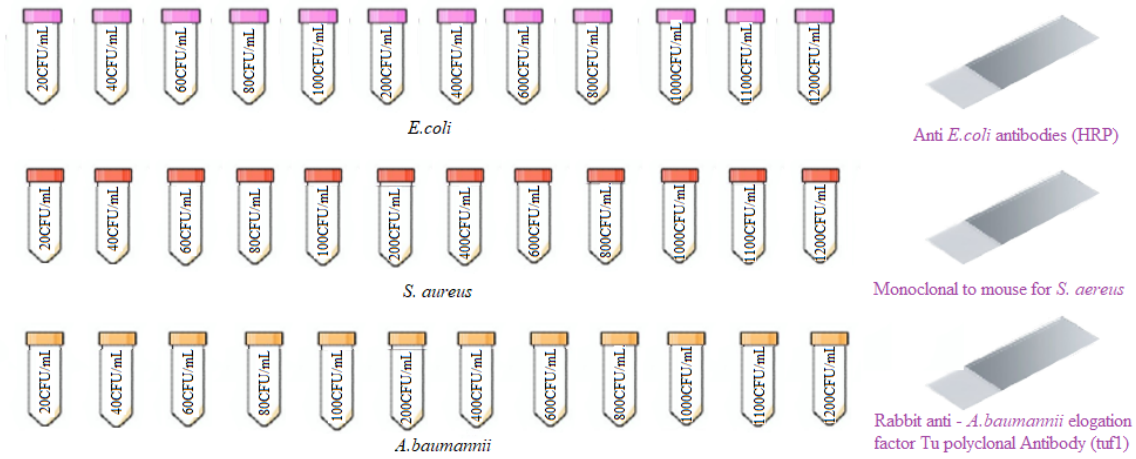

Figure 12. Determination of Linear Range and Analytical Sensitivity (Limit Of Detection)

4. RESULTS & DISCUSSION

4.1 Glass Surface Modification And Aptamer/Antibody Immobilization

The chosen aptamers were attached to the altered glass surfaces during the aptamer immobilization procedure, as previously mentioned. After the immobilization, cleaning procedures were carried out to clean the surfaces of any unbound aptamers. Spectroscopic techniques were used to determine the aptamer concentrations in the washing solutions that included the unbound aptamers. This was accomplished by using a spectrophotometer to measure the absorbance of the washing solutions at various wavelengths. Similar to this, the antibodies were bonded to the altered glass surfaces during the antibody immobilization procedure. The unbound antibodies were removed by wash procedures and the washing solutions containing the unbound antibodies were collected. To measure the antibody concentrations in the washing solutions, spectroscopic techniques were used to measure the absorbance at specific wavelengths, and the obtained data were then used to calculate the percentages of antibody binding. Quantitative measurements of aptamer and antibody concentrations obtained as a result of the spectroscopic techniques used were used to calculate binding percentages and evaluate the effectiveness of immobilization procedures.

As a success criterion, we established that aptamers should have an immobilization efficiency of at least 80%. We found that the success criterion for antibodies should be a minimum immobilization effectiveness of 90%. In order to fulfill the success criteria, we established, we reached the necessary immobilization efficiency levels and changed the incubation times. We validate the efficacy of the immobilization procedure, measure the concentration of aptamers and antibodies in the wash solutions. We obtained the binding percentages and used an equation to compute the binding efficiency. The aptamer immobilization efficiency analysis led us to the conclusion that binding efficiency was not significantly affected by incubation durations more than 30 minutes. As a result, a 30 minutes incubation period was chosen for aptamer immobilization. It was discovered that incubation intervals longer than 25 minutes did not substantially improve the binding effectiveness for the antibody immobilization investigations. As a result, a 25-minute incubation period was chosen for antibody immobilization.

4.2 Efficiency Of Aptamer/Antibody Immobilization

The incubation period was optimized and found to be 30 minutes for aptamers to reach a minimum immobilization effectiveness of 80%. The measurement results for aptamer immobilization are shown in Figure 13 and Table 2, and it was shown that extending the incubation period over 30 minutes did not significantly increase the binding efficiency.

Similarly, a minimum immobilization effectiveness of 90% was desired for antibody immobilization. Due to the fact that subsequent increases did not result in appreciable gains in binding efficiency, the incubation period was tuned and set at 25 minutes. Figure 14 and Table 2 show the measurement findings for antibody immobilization, respectively.

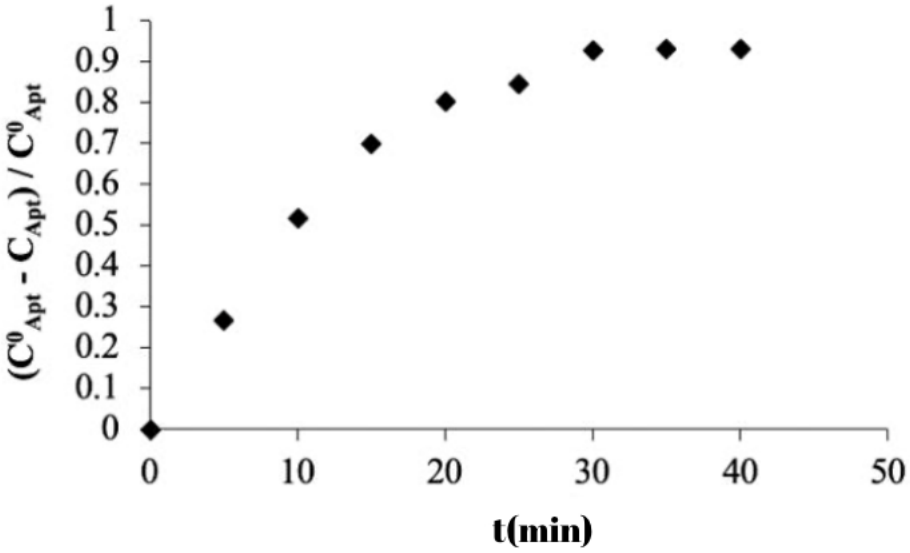

Figure 13. Efficiency of Aptamer Immobilization

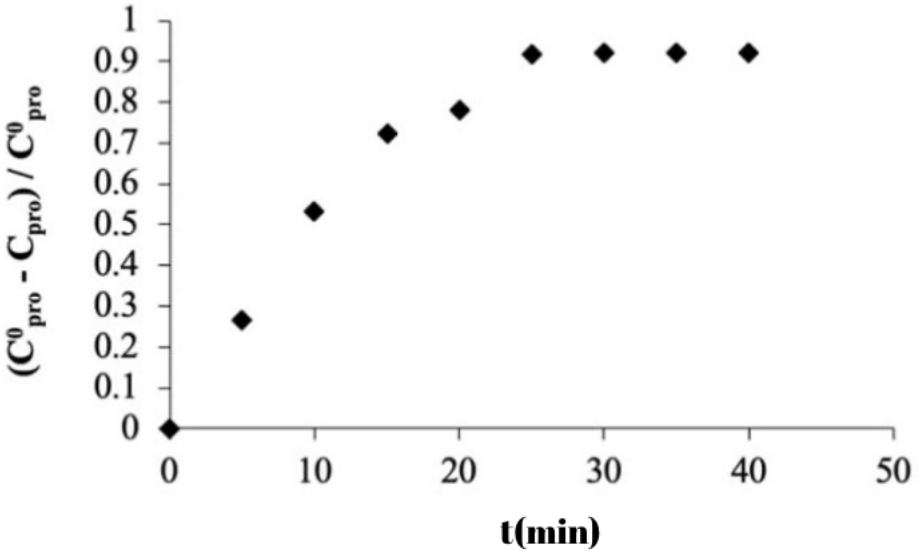

Figure 14. Efficiency of Antibody Immobilization

Table 2. Efficiency of Aptamer/Antibody Immobilization

APTAMER		**ANTIBODY**	
t [min.]	**$(C_0-C) / C_0$**	**t [min.]**	**$(C_0-C) / C_0$**
0	0	**0**	0
5	0.26	**5**	0.26
10	0.51	**10**	0.53
15	0.69	**15**	0.72
20	0.80	**20**	0.78
25	0.84	**25**	0.91
30	0.92	**30**	0.91
35	0.92	**35**	0.92
40	0.93	**40**	0.92

4.3 Reproducibility Of Ligand Immobilization

To evaluate the repeatability of ligand immobilization, replication tests including several operators, production batches, and time points were carried out. The repeatability was examined using the coefficient of variation, which was computed using the mean and standard deviation data. Results from 96 immobilizations showed a standard deviation of 1.60 σ, an average immobilization efficiency of 92.2%, and a coefficient of variation of 1.74 σ (Table 3). In aptamer immobilizations, higher coefficients of variation were observed (3.15 σ for EL-5 and for 3.36 σ SL-5), along with higher average immobilization efficiency (93 % for EL-5 and 93 5% for SL-5), compared to other ligand immobilizations (APX. Table 3).

For antibody immobilization, the success requirements for immobilization effectiveness were established at a minimum of 90%, and for aptamer immobilization, a minimum of 80%. The study was completed after 96 immobilization experiments, containing 12 ligands and 8 repetitions, were completed. This investigation showed a 90.6% success rate for immobilization, with 87 out of 96 immobilizations meeting the success criterion.

Table 3. Reproducibility of Ligand Immobilization

	Replica	**Standard Deviation (σ)**	**Average Immobilization Efficiency (%)**	**Coefficients of Variation (%)**
EL-1	8	1.56	92.0	1.69
EL-2	8	1.67	92.8	1.80
EL-3	8	1.72	92.0	1.87
EL-4	8	1.64	92.1	1.79
EL-5	8	3.15	93.0	3.39
SL-1	8	1.64	92.0	1.78
SL-2	8	1.72	92.8	1.85
SL-3	8	1.59	92.0	1.73
SL-4	8	1.64	92.9	1.77
SL-5	8	3.36	93.0	3.62

AL-1	8	1.60	91.0	1.76
AL-2	8	1.72	91.1	1.88
Total	96	1.60	92.2	1.74

In APX Table 3, the comprehensive findings of the repeated trials for all ligands in terms of ligand immobilization were shown, highlighting the study's repeatability. For each ligand, the immobilization procedure was carried out many than thrice, and the results were meticulously recorded and examined. The acquired findings repeatedly showed that the ligands were successfully immobilized on the targeted surfaces, guaranteeing the validity and repeatability of the experimental method.

4.4 Determination Of Ligands With High Affinity To The Target Bacterium And Not Cross-Reacting

Each of the 12 ligands was immobilized on a total of 36 glass surfaces, working in triplicate for each bacterial species independently, in order to find ligands with a high affinity for the target bacteria and no cross-reactivity. At least 80% of the aptamer and 90% of the antibody could be immobilized on the glass surfaces employed in this work. The effectiveness of the ligands was then assessed using a calculation of bacteria capture efficiency.

Based on the results of the bacteria capture efficiency, the following ligands were selected: Anti-*E. coli* antibody (HRP) (ab68450) for *E. coli*, Mouse monoclonal to S. aureus for *S. aureus*, and Rabbit anti - *A. baumannii* elongation factor Tu polyclonal Antibody (tuf1) for *A. baumannii* (Table 4).

The Anti-*E. coli* antibody (HRP) exhibited a capture efficiency of 89.1% for *E. coli*, while capturing only 1.2% and 1.1% for other bacteria. This indicates that the use of Anti-*E. coli* antibody (HRP) for specific detection was highly successful. The Mouse monoclonal to *S. aureus* showed a capture efficiency of 88.2% for *S. aureus* and bound only 1.1% and 1.0% to other bacteria. Therefore, it proved to be an effective ligand for the specific detection of *S. aureus*. Lastly, the Rabbit anti - *A. baumannii* elongation factor Tu polyclonal Antibody (tuf1) demonstrated a capture efficiency of 86.1% for *A. baumannii* and captured 2.2% and

2.2% for other bacteria. This suggested that it could be a suitable ligand for detection purposes.

As a result, these selected ligands showed great capture efficiency and affinity for the target species, while showing low capture efficiency for other bacteria. This indicates that they can identify target microorganisms with precision and efficiency.

Table 4. Determination of Ligands with High Affinity to the Target Bacterium

	Glass Surface	**Bacteria Capture Efficiency (%)**		
Ligands	**Antibody/Aptamer**	***E. coli***	***S. aureus***	***A. baumannii***
E. coli	Anti-*E. coli* antibody - Abcam (ab25823)	84.2	3.1	3.0
	Rabbit polyclonal antibodies-Serotec (4329-4906)	78.9	4.2	3.9
	Anti-*E. coli* antibodies (HRP) - Abcam (ab68450)	89.1	1.2	1.1
	Anti- *E. coli* LPS antibodies – Abcam (ab35654)	79.5	4.2	3.9
	Aptamer	85.3	2.1	1.9
S. aureus	Mouse monoclonal [704] to *S. Aureus*- Abcam (ab37644)	3.1	83.2	3.0
	Anti-*S. aureus* Enterotoxins A + B + TSST-1 antibody- Abcam (ab190337)	3.2	83.0	3.0
	Anti-*S. aureus* antibody- Abcam (ab20920)	4.3	78.6	3.8
	Mouse monoclonal [702] to *S. aureus*- Abcam (ab20002)	1.1	88.2	1.0
	Aptamer	2.3	85.5	2.2
	Rabbit anti - *A. baumannii* elongation factor Tu polyclonal	2.2	2.2	86.1

A. baumannii	Antibody (tuf1) - MYBIOSOURCE (MBS1491803)			
	Rabbit anti - *A. baumannii* elongation factor Tu polyclonal Antibody (tuf1). Biotin conjugated -MYBIOSOURCE (MBS1492257)	4.3	4.2	81.4

4.5 Immobilization Of Selected Ligands And Reproducibility

The experiment benefited from an optimized incubation time of 25 minutes. The amounts of antibodies in the washing solutions were examined using spectroscopic techniques to calculate the percentages of antibody binding. In order to evaluate the trapping efficiency of various bacteria, we created six solutions with concentrations of 100, 200, 400, 600, 800 and 1000 CFU/mL for each of the three bacteria. The study utilized 288 different glass substrates in all. In total, 288 glass substrates were used in the study. Specifically, 96 glass substrates were modified with Anti-*E. coli* antibodies (HRP) for *E. coli*, another 96 glass substrates were modified with mouse monoclonal antibodies specific to *S. aureus* for *S. aureus*, and the remaining 96 glass substrates were modified with rabbit anti-*A. baumannii* Elongation factor Tu polyclonal Antibody (tuf1) for *A. baumannii*. These substrates were then used to analyze the capture efficiency of the respective bacteria (Table 5).

The binding efficiency was calculated using the formula $[(C^0_{pro} – C_{pro}) / C^0_{pro}]$, where $[C^0_{pro}]$ and $[C_{pro}]$ represent the protein concentrations, allowing for the calculation of the binding efficiency. The linear range and analytical sensitivity (limit of detection) experiments were conducted on glass surfaces with at least 90% antibody immobilization efficiency. The bacteria capture efficiency was defined as the ratio of the bacteria concentration at the outlet to the concentration provided at the inlet, minus 1.0. By using the values of $[C^0_{bac}]$ and $[C_{bac}]$ for the bacteria capture efficiency calculations, the ratio of the solid-phase bacteria concentration to the initial bacterial concentration was determined through the $[(C^0_{bac} - C_{bac}) / C^0_{bac}]$ calculation. This allowed for the calculation of bacteria capture efficiency.

The coefficients of variation were computed using the mean and standard deviation values of the determined bacteria capture efficiency percentages in order to assess the experiment's repeatability. The percent coefficients of variation were determined as 5.21 for *E. coli*, 5.01 for *S. aureus*, and 3.89 for *A. baumannii*. These numbers represent the variability of the experiment. However, all three bacteria met the desired success conditions, with variation coefficient values around 10% (APX. Table 4).

The complete results and information on the immobilization of certain ligands and the study's repeatability may be found in (APX. Table 4). The immobilization procedure is thoroughly examined in this table, along with the particular ligands utilized, the immobilization technique used, and the resulting results. The statistics show that the immobilization process is consistent and repeatable, which is essential for guaranteeing the accuracy of future studies and measurements.

Table 5. Immobilization of Selected Ligands and Reproducibility

Bacteria	**Concentration (CFU/mL)**	**Replica**	**Standard Deviation**	**Average Bacteria Capture Efficiency (%)**	**Coefficient of Variation (%)**
E. coli	100	16	3.28	90.1	3.64
	200	16	3.21	90.1	3.57
	400	16	4.02	89.1	4.52
	600	16	4.05	89.1	4.55
	800	16	4.61	88.1	5.24
	1000	16	4.65	83.1	5.60
	Total	96	4.60	88.3	5.21
S. aureus	100	16	3.15	88.9	3.54
	200	16	3.30	89.0	3.71
	400	16	4.02	88.0	4.56
	600	16	4.00	88.0	4.54
	800	16	4.74	87.2	5.44

	1000	16	4.58	83.2	5.51
	Total	96	4.38	87.4	5.01
A. baumannii	100	16	2.17	87.0	2.49
	200	16	2.08	87.0	2.40
	400	16	2.82	86.0	3.28
	600	16	2.85	86.0	3.32
	800	16	3.81	85.3	4.47
	1000	16	3.62	82.1	4.41
	Total	96	3.33	85.6	3.89

4.6 Linear Range And Analytical Sensitivity (Limit Of Detection)

Here, we discuss research aimed at creating a dependable, affordable biosensor for identifying bacterial infections in sepsis patients. The biosensor was designed using antibodies as ligands to specifically capture and detect three types of bacteria: *E. coli*, *S. aureus,* and *A. baumannii.* The study involved modifying 144 glass surfaces for each of the bacteria, resulting in a total of 432 surfaces. The efficiency of the biosensor was evaluated using tests on the modified surfaces for linear range and analytical sensitivity (detection limit).

Bacteria capture efficiency was determined by calculating the ratio of the bacteria concentration at the outlet to the concentration provided at the inlet, subtracting 1.0 from the result. The calculation $[(C^0_{bac}–C_{bac})/C^0_{bac}]$ was used to determine the bacteria capture efficiency, where $[C^0_{bac}]$ represents the initial bacterial concentration and $[C_{bac}]$ represents the solid-phase bacteria concentration. This computation was done many times with doses of each bacterium ranging from 20 to 1200 CFU/mL. Figures 15-16-17 show the link between the liquid-phase bacterial concentration and the solid-phase bacterial count using the computed bacterial counts.

The bacteria capture efficiency was measured for each solid-phase bacterial count using multiple runs at concentrations of 20, 40, 60, 80, 100, 200, 400, 600, 800, 1000, and 1200 CFU/mL in order to examine the linear range and analytical sensitivity. The quantity

of bacteria that were collected was counted, and a graph showing the link between the quantity of solid-phase bacteria and the quantity of liquid-phase bacteria was produced. The outcomes are shown in APX.TABLE.4.

For each of the three bacteria, 12 solutions at concentrations were made for the linear range and analytical sensitivity investigation. A total of 432 glass surfaces were used, with 144 surfaces modified with Anti-*E. coli* antibodies (HRP) for *E. coli*, 144 surfaces modified with mouse monoclonal to *S. aureus* antibodies for *S. aureus*, and 144 surfaces modified with rabbit anti-*A. baumannii* Elongation factor Tu polyclonal Antibody (tuf1) for *A. baumannii*. The linear range and analytical sensitivity studies were conducted on these glass surfaces.

The analytical sensitivity (detection limit) of the device was determined by the observation that the bacterial capture efficiency decreased at concentrations below 100 CFU/mL. It was discovered that at high bacterial concentrations, the ligands that capture the bacteria might become saturated, which would lead to a decline in the average bacterial capture efficiency at 1000 CFU/mL. Only repeated data within this concentration range were utilized to construct graphs showing the association between solid-phase bacteria count and liquid-phase bacteria concentration in order to assure consistent results (Figures 18-19-20). Measurements outside the concentration range of 100-1000 CFU/mL were omitted. The correlation coefficient values were calculated as 0.98 for *E. coli*, 0.98 for *S. aureus*, and 0.99 for *A. baumannii*, indicating that the developed system produced directly proportional results within the concentration range of 100-1000 CFU/mL.

Solid-phase bacteria count against liquid-phase bacteria concentration graphs were created as a result, showing that the proposed method had a high level of linearity and proportionality within this range by omitting readings outside the 100-1000 CFU/mL concentration range. The correlation coefficient values further confirmed the reliability of the system in the concentration range of 100-1000 CFU/mL for *E. coli, S. aureus,* and *A. baumannii.*

We discovered that the biosensor had an analytical sensitivity of 100 CFU/ml and could detect bacteria at concentrations higher than 100 CFU/ml. The low bacterial density clinging to the surface, which leads to a significant loss of concentration after extracting the

bacteria from the surface, may be the cause of the failure to deliver reliable findings below the detection limit of 100 CFU/mL. The reading equipment may not be very effective at picking up germs with low concentrations, which might account for this. Even if surface immobilization is effectively accomplished, the device is susceptible to mistakes during the reading process due to the low turbidity caused by the removed bacteria from the surface.

We might be able to guarantee the performance of our system for bacterial concentrations below 100 CFU/mL by using a device capable of measuring lower bacterial concentrations with better sensitivity. However, at high bacterial concentrations, ligands that trapped bacteria may become saturated, which would reduce the efficacy of bacterial capture.

In order to create graphs with a high correlation coefficient, values outside the range of 100-1000 CFU/ml were omitted from the study and the solid-phase bacterial count was plotted against the liquid-phase bacterial concentration.

In a research employing optical density, comparable to our method, Rajnovic at all [47] achieved quick detection. They attempted to minimize the incubation time in their phage detection-based system and were successful in doing so, cutting it down to as low as 45 minutes for analysis. With contrast, with our designed system, we were able to outperform Rajnovic in terms of analysis time by using incubation periods of just 25 and 30 minutes. While we were able to effectively identify bacteria even at a concentration of 100 CFU/mL with only a 30-minute incubation, Wang at all [48], was able to detect bacteria at a concentration of 105 CFU/mL within 4 hours of their investigation.

Table 6. Variation of Bacteria Capture Efficiency with Concentration

Concentration (CFU/mL)	**Replica**	***E. coli***	***S. aureus***	***A. baumannii***
		Average Bacteria Capture Efficiency (%)		
20	8	1.0	1.2	1.0
40	8	1.9	2.1	2.3
60	8	14.7	13.1	12.5
80	8	40.6	36.9	35.4
100	16	90.1	88.9	87.0

200	16	90.1	89.0	87.0
400	16	89.1	88.0	86.0
600	16	89.1	88.0	86.0
800	16	88.1	87.2	85.3
1000	16	83.1	83.2	82.1
1100	8	75.7	75.8	75.1
1200	8	69.1	69.3	68.5

Table 7. Variation of Solid Phase Bacteria Number with Concentration

Concentration (CFU/mL)	***E. coli***	***S. aureus***	***A. baumannii***
	Solid Phase Bacteria Count		
20	0	0	0
40	1	1	1
60	9	8	8
80	32	30	28
100	90	89	87
200	180	178	174
400	356	352	344
600	534	528	516
800	704	697	682
1000	831	832	821
1100	833	833	826
1200	829	832	821

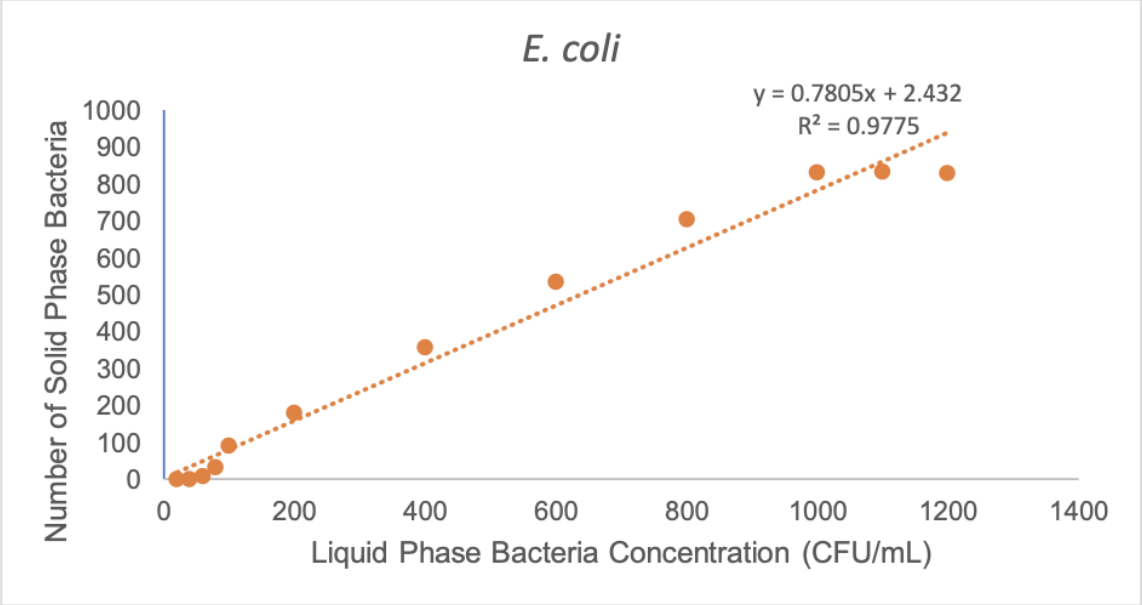

Figure 15. Efficiency of Bacterial Capture as A Function of Liquid Phase Bacterial Concentration and Solid Phase Bacterial Count for *E. coli*

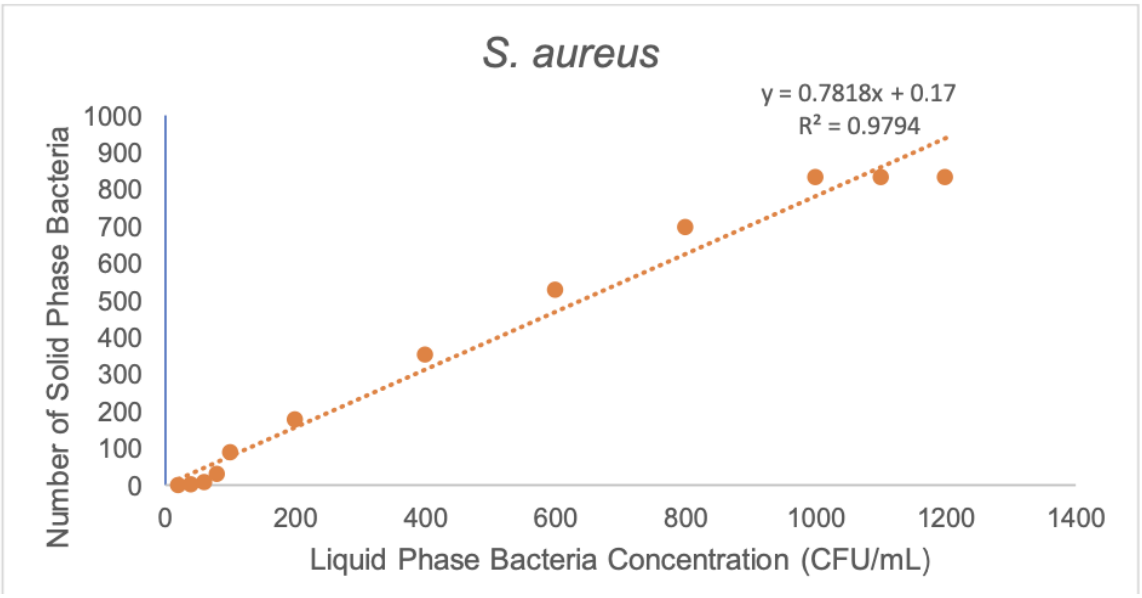

Figure 16. Efficiency of Bacterial Capture as A Function of Liquid Phase Bacterial Concentration and Solid Phase Bacterial Count for *S. aureus*

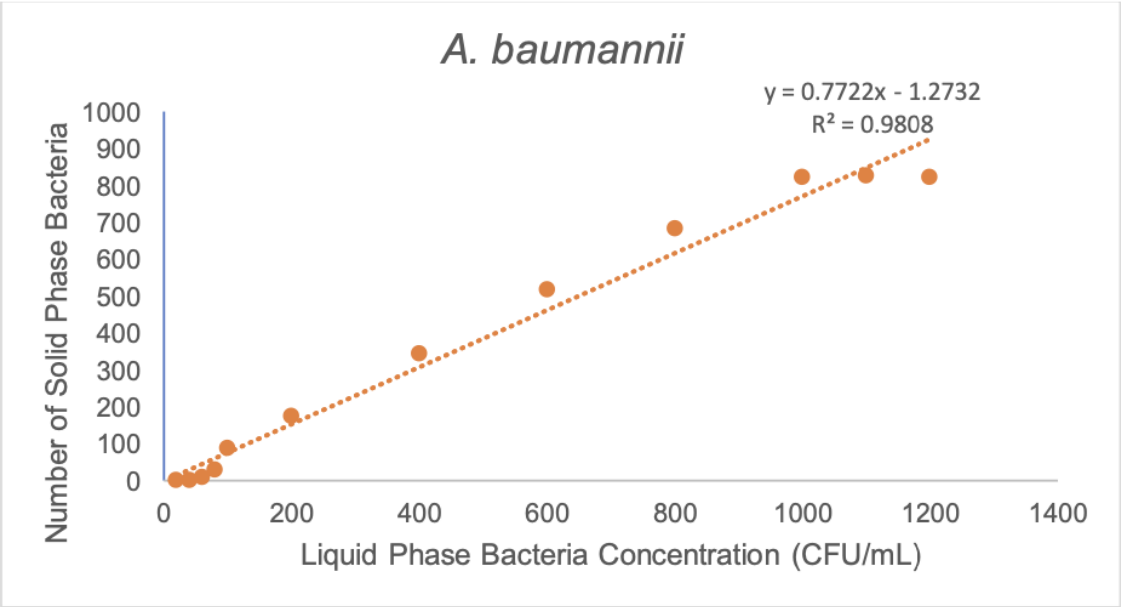

Figure 17. Efficiency of Bacterial Capture as A Function of Liquid Phase Bacterial Concentration and Solid Phase Bacterial Count for *A. baumannii*

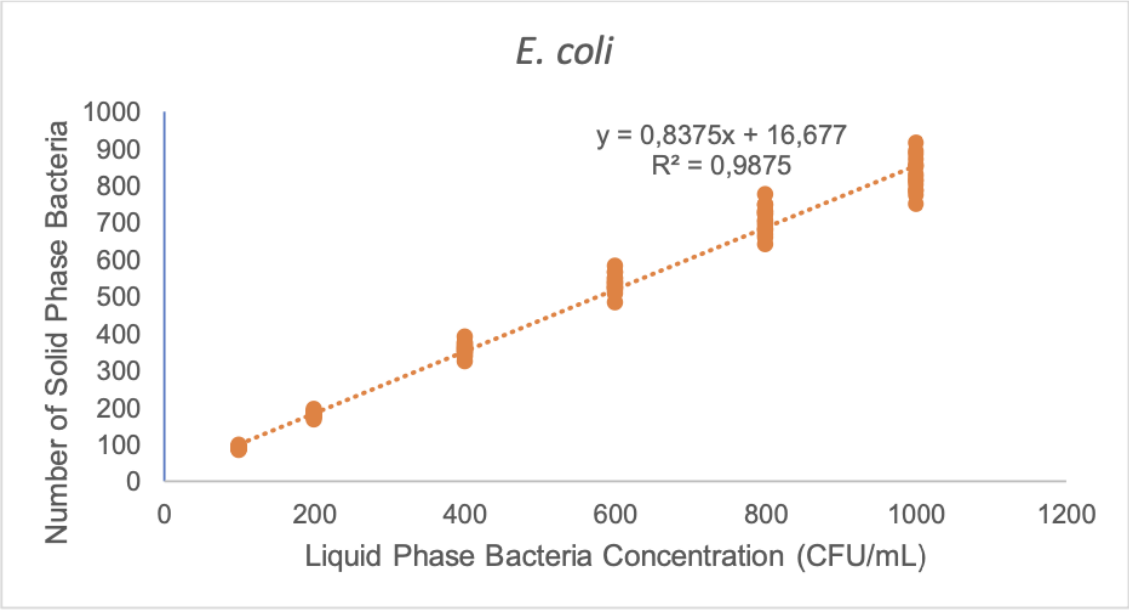

Figure 18. Efficiency of Bacterial Capture for *E. coli* Excluding Measurements Outside the Concentration Range

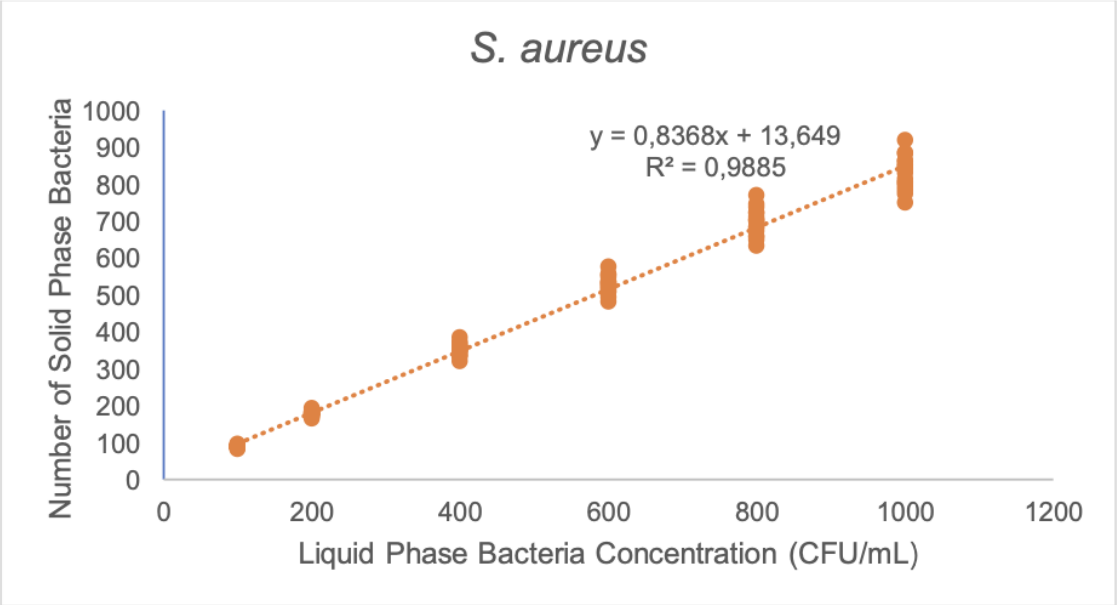

Figure 19. Efficiency of Bacterial Capture for *S. aureus* Excluding Measurements Outside the Concentration Range

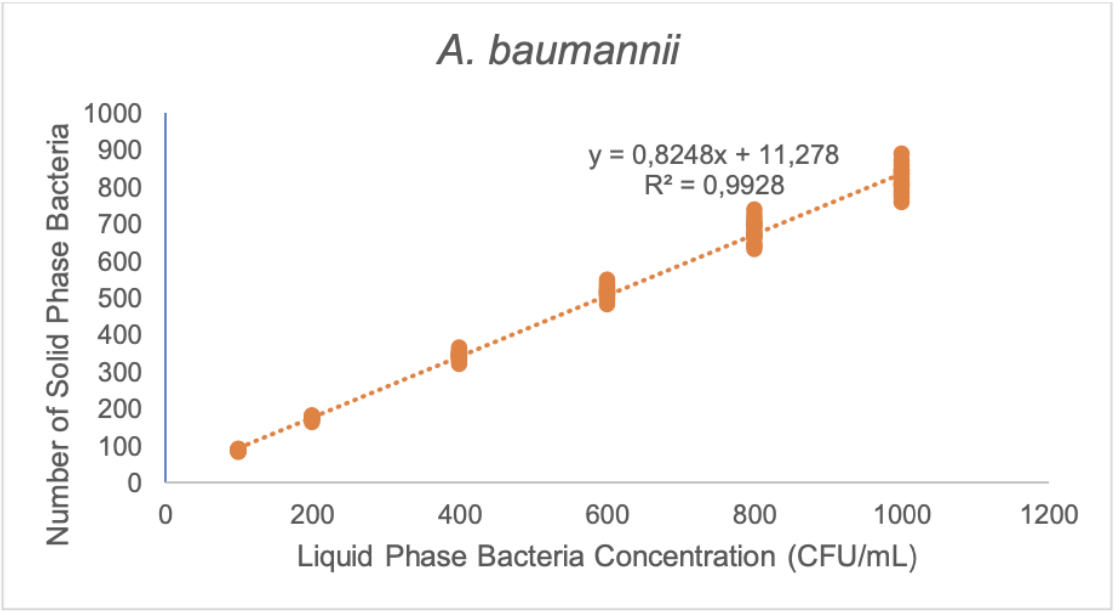

Figure 20. Efficiency of Bacterial Capture for *A. baumannii* Excluding Measurements Outside the Concentration Range

As a result, we found in our work that antibodies are still more accurate ligands for identifying sepsis pathogens than aptamers. The ligands used for the investigation exhibited a high affinity for the target bacterium and showed no cross-reactivity. The biosensor produced consistent, reproducible, linear, and analytically sensitive results. To build a

biosensor that might identify bacterial infections in sepsis patients, we discovered suitable ligands.

In addition to all of this, a crucial note about aptamers' advantages over antibodies in the creation of biosensors should not be overlooked. Despite the fact that aptamers were successful in this study, where they attained an efficiency of 85%, it is crucial to take into account the great potential of aptamers. Antibodies are recognized as dependable ligands. Compared to antibodies, aptamers have a number of benefits. They can be easily made and changed, have excellent specificity and binding affinity for their target molecules, and are reduced in size, allowing for easier access to the target. Aptamers are more resilient because they exhibit good stability under a range of environmental variables, including temperature, pH, and salt concentration. Additionally, they provide excellent repeatability and are more economical. These characteristics make aptamers highly promising for their use in biosensors.

We have found appropriate ligands for the creation of low-cost, highly sensitive biosensors based on the results that we have acquired. Furthermore, we have shown that aptamers have better potential. We can investigate the creation of aptasensors employing aptamer ligands in our future research, which may present new opportunities. We can safely claim, based on the results of these investigations, that we can use these methodologies to discover suitable ligands for the creation of biosensors to detect additional illnesses. For the purpose of identifying certain infections, we can also do further analyses. Our work could result in the creation of affordable, superior biosensors.

5. CONCLUSION

In this research, we examined the efficacy of aptamer and antibody ligands for detecting harmful bacteria on glass surfaces. Through systematic experiments conducted on the pathogens most commonly associated with sepsis (*Escherichia coli, Staphylococcus aureus,* and *Acinetobacter baumannii),* we evaluated the potential of both antibody and aptamer-based biosensors. The comparative analysis of 12 ligands revealed that anti-*E. coli* antibody (HRP) for *E. coli*, mouse monoclonal to *S. aureus* for *S. aureus*, and Elongation factor Tu polyclonal Antibody (tuf1) rabbit anti-*A. baumannii* for *A. baumannii* demonstrated superior performance.

Additionally, we verified the effectiveness of the top-performing ligands by doing additional research on the linear range, analytical sensitivity, and reproducibility of findings. The detection threshold was set at 100 CFU/mL. Within the concentration range of 100 CFU/mL to 1000 CFU/mL, the device was confirmed to deliver accurate findings. The performance of the system we created was verified with a coefficient of variation (CV) of less than 5.5%. The antibody ligands still outperformed the aptamer ligands in terms of efficiency. In contrast, our results show that the use of aptamer ligands has great potential.

In this thesis, we presented guiding information for ligand selection, especially in the creation of biosensors for the detection of bacterial infections. The selected ligands can be used as important resources for further studies and the creation of low-cost, high-sensitivity biosensor systems. Aptamer-based biosensors may be further developed and improved to increase their analytical capabilities and broaden their applications in the field of pathogen detection.

Overall, this work emphasizes the significance of ligand choice and offers information on the effectiveness of aptamer- and antibody-based biosensors for bacterial detection. Additional study in this field may lead to improvements in diagnostic methods and aid in the creation of ground-breaking treatments for bacterial disease.

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APPENDICES

Appendix 1: Chemicals

Apx.Table 1. Chemicals

Glycidoxypropyl-trimethoxysilane	Sigma-Aldrich (440167)
Acetone	Sigma-Aldrich (539481)
Ether	Sigma-Aldrich (271454)
Carbonyldiimidazole	Sigma-Aldrich (115533)
Acetonitrile	Sigma-Aldrich (271004)
Sodium phosphate solution	Sigma-Aldrich (74092)
Phosphate-buffered saline	Sigma-Aldrich (P4417)
Ethanolamine solution	Sigma-Aldrich (398136)
Ethidium bromide	Sigma-Aldrich (E7637)
Amino silane solution	Sigma-Aldrich (440140)
3%-aminopropyltriethoxysilane solution	Thermo Scientific (80370)
Glutaraldehyde solution	Sigma-Aldrich (3802)
Tween-20	Sigma-Aldrich (P9416)
Muller Hinton Agar	Sigma-Aldrich (70191)
Columbia Agar	Sigma-Aldrich (27688)
LB Broth	Sigma-Aldrich (L2542)

Appendix 2: Equipment

Apx.Table 2. Equipment

Incubator	Panasonic (PHCbi MCO-230AICUVL-PA)
Orbital Shaker	BIOSAN (PSU-10I)
Spectrophotometer	Denovix (DS-11 FX+)
Quartz cuvette	ISOLAB (09801001)
Centrifuge	BIOSAN (LMC4200R)
Magnetic stirrer	DAIHAN (MS-A)
Microcentrifuge	Thermo Scientific (MicroCL 21)
Precision balance	KERN (PCB 1000-2)
LP Vortex mixer	Thermo Scientific (88880017)
Mini Centrifuge	Thermo Scientific (mySPIN™ 6)

Appendix 3: Reproducibility of Ligand Immobilization

Apx.Table 3. Reproducibility Of Ligand Immobilization

	Immobilization Efficiency (%)		Immobilization Efficiency (%)		Immobilization Efficiency (%)
EL-1-R1	92.2	**EL-5-R1**	93.7	**SL-4-R1**	92.5
EL-1-R2	89.1	**EL-5-R2**	95.5	**SL-4-R2**	96.0
EL-1-R3	91.0	**EL-5-R3**	86.3	**SL-4-R3**	94.7
EL-1-R4	92.7	**EL-5-R4**	97.0	**SL-4-R4**	90.3
EL-1-R5	94.0	**EL-5-R5**	93.8	**SL-4-R5**	93.6
EL-1-R6	92.0	**EL-5-R6**	93.8	**SL-4-R6**	92.3
EL-1-R7	91.5	**EL-5-R7**	91.8	**SL-4-R7**	91.6
EL-1-R8	93.7	**EL-5-R8**	92.6	**SL-4-R8**	92.7
EL-2-R1	93.1	**SL-1-R1**	92.5	**SL-5-R1**	97.9
EL-2-R2	90.4	**SL-1-R2**	89.3	**SL-5-R2**	93.4
EL-2-R3	92.5	**SL-1-R3**	90.3	**SL-5-R3**	86.4
EL-2-R4	91.3	**SL-1-R4**	92.3	**SL-5-R4**	93.5
EL-2-R5	92.6	**SL-1-R5**	92.5	**SL-5-R5**	95.7
EL-2-R6	92.5	**SL-1-R6**	94.0	**SL-5-R6**	93.8
EL-2-R7	94.2	**SL-1-R7**	94.0	**SL-5-R7**	91.2
EL-2-R8	95.9	**SL-1-R8**	91.5	**SL-5-R8**	92.6
EL-3-R1	94.8	**SL-2-R1**	92.1	**AL-1-R1**	91.5
EL-3-R2	90.0	**SL-2-R2**	95.7	**AL-1-R2**	88.3
EL-3-R3	92.3	**SL-2-R3**	92.5	**AL-1-R3**	91.3
EL-3-R4	93.3	**SL-2-R4**	90.5	**AL-1-R4**	93.2
EL-3-R5	92.8	**SL-2-R5**	94.7	**AL-1-R5**	89.5
EL-3-R6	89.4	**SL-2-R6**	93.8	**AL-1-R6**	92.6
EL-3-R7	91.8	**SL-2-R7**	91.4	**AL-1-R7**	90.3
EL-3-R8	92.3	**SL-2-R8**	92.6	**AL-1-R8**	91.6
EL-4-R1	89.5	**SL-3-R1**	89.4	**AL-2-R1**	88.4
EL-4-R2	92.1	**SL-3-R2**	92.5	**AL-2-R2**	89.2

EL-4-R3	92.3	**SL-3-R3**	92.4	**AL-2-R3**	93.9
EL-4-R4	94.5	**SL-3-R4**	90.2	**AL-2-R4**	92.2
EL-4-R5	90.3	**SL-3-R5**	94.1	**AL-2-R5**	91.5
EL-4-R6	91.9	**SL-3-R6**	93.7	**AL-2-R6**	91.8
EL-4-R7	92.5	**SL-3-R7**	91.4	**AL-2-R7**	90.5
EL-4-R8	93.9	**SL-3-R8**	92.6	**AL-2-R8**	91.6

Appendix 4: Immobilization of Selected Ligands And Reprocubility

Apx.Table 4. Immobilization of Selected Ligands and Reproducibility

Concentration	Efficiency (%)	Concentration	Efficiency (%)	Concentration	Efficiency (%)
***E. coli* 20 R1**	0.9	***S. aureus* 20 R1**	0.9	***A. baumannii* 20 R1**	0.2
***E. coli* 20 R2**	1.2	***S. aureus* 20 R2**	1.0	***A. baumannii* 20 R2**	0.2
***E. coli* 20 R3**	1.3	***S. aureus* 20 R3**	1.0	***A. baumannii* 20 R3**	1.3
***E. coli* 20 R4**	0.7	***S. aureus* 20 R4**	1.5	***A. baumannii* 20 R4**	1.4
***E. coli* 20 R5**	0.9	***S. aureus* 20 R5**	2.3	***A. baumannii* 20 R5**	1.9
***E. coli* 20 R6**	0.9	***S. aureus* 20 R6**	0.9	***A. baumannii* 20 R6**	2.3
***E. coli* 20 R7**	1.1	***S. aureus* 20 R7**	1.0	***A. baumannii* 20 R7**	0.3
***E. coli* 20 R8**	1.2	***S. aureus* 20 R8**	0.9	***A. baumannii* 20 R8**	0.3
***E. coli* 40 R1**	1.4	***S. aureus* 40 R1**	2.3	***A. baumannii* 40 R1**	2.1
***E. coli* 40 R2**	1.9	***S. aureus* 40 R2**	2.7	***A. baumannii* 40 R2**	2.6
***E. coli* 40 R3**	2.1	***S. aureus* 40 R3**	1.9	***A. baumannii* 40 R3**	1.9
***E. coli* 40 R4**	2.5	***S. aureus* 40 R4**	1.3	***A. baumannii* 40 R4**	1.2
***E. coli* 40 R5**	2.1	***S. aureus* 40 R5**	1.9	***A. baumannii* 40 R5**	2.3
***E. coli* 40 R6**	1.5	***S. aureus* 40 R6**	2.5	***A. baumannii* 40 R6**	3.2
***E. coli* 40 R7**	2.0	***S. aureus* 40 R7**	3.0	***A. baumannii* 40 R7**	2.7
***E. coli* 40 R8**	1.6	***S. aureus* 40 R8**	1.0	***A. baumannii* 40 R8**	2.3
***E. coli* 60 R1**	13.5	***S. aureus* 60 R1**	13.2	***A. baumannii* 60 R1**	12.3
***E. coli* 60 R2**	12.8	***S. aureus* 60 R2**	12.9	***A. baumannii* 60 R2**	12.9
***E. coli* 60 R3**	15.6	***S. aureus* 60 R3**	13.5	***A. baumannii* 60 R3**	13.4
***E. coli* 60 R4**	15.4	***S. aureus* 60 R4**	13.7	***A. baumannii* 60 R4**	11.7
***E. coli* 60 R5**	16.1	***S. aureus* 60 R5**	13.2	***A. baumannii* 60 R5**	11.6
***E. coli* 60 R6**	14.7	***S. aureus* 60 R6**	12.6	***A. baumannii* 60 R6**	12.6
***E. coli* 60 R7**	14.5	***S. aureus* 60 R7**	12.8	***A. baumannii* 60 R7**	12.7
***E. coli* 60 R8**	15.0	***S. aureus* 60 R8**	13.0	***A. baumannii* 60 R8**	12.9
***E. coli* 80 R1**	38.5	***S. aureus* 80 R1**	37.2	***A. baumannii* 80 R1**	35.4
***E. coli* 80 R2**	39.3	***S. aureus* 80 R2**	37.3	***A. baumannii* 80 R2**	35.1
***E. coli* 80 R3**	42.3	***S. aureus* 80 R3**	36.9	***A. baumannii* 80 R3**	33.4
***E. coli* 80 R4**	43.2	***S. aureus* 80 R4**	36.3	***A. baumannii* 80 R4**	34.6

***E. coli* 80 R5**	39.4	***S. aureus* 80 R5**	35.2	***A. baumannii* 80 R5**	36.5
***E. coli* 80 R6**	47.3	***S. aureus* 80 R6**	36.9	***A. baumannii* 80 R6**	36.4
***E. coli* 80 R7**	33.4	***S. aureus* 80 R7**	37.5	***A. baumannii* 80 R7**	36.3
***E. coli* 80 R8**	41.0	***S. aureus* 80 R8**	38.0	***A. baumannii* 80 R8**	35.0
E. coli 100 R1	88.0	***S. aureus* 100 R1**	88.2	***A. baumannii* 100 R1**	87.3
***E. coli* 100 R2**	88.8	***S. aureus* 100 R2**	89.1	***A. baumannii* 100 R2**	87.3
***E. coli* 100 R3**	90.1	***S. aureus* 100 R3**	88.3	***A. baumannii* 100 R3**	86.3
***E. coli* 100 R4**	90.3	***S. aureus* 100 R4**	89.2	***A. baumannii* 100 R4**	87.4
***E. coli* 100 R5**	83.2	***S. aureus* 100 R5**	82.1	***A. baumannii* 100 R5**	82.0
***E. coli* 100 R6**	85.8	***S. aureus* 100 R6**	85.3	***A. baumannii* 100 R6**	84.2
***E. coli* 100 R7**	87.4	***S. aureus* 100 R7**	85.2	***A. baumannii* 100 R7**	85.3
***E. coli* 100 R8**	92.2	***S. aureus* 100 R8**	87.8	***A. baumannii* 100 R8**	86.2
***E. coli* 100 R9**	93.2	***S. aureus* 100 R9**	91.2	***A. baumannii* 100 R9**	87.2
***E. coli* 100 R10**	94.0	***S. aureus* 100 R10**	91.3	***A. baumannii* 100 R10**	87.3
***E. coli* 100 R11**	97.2	***S. aureus* 100 R11**	93.2	***A. baumannii* 100 R11**	86.4
***E. coli* 100 R12**	89.9	***S. aureus* 100 R12**	95.4	***A. baumannii* 100 R12**	87.1
***E. coli* 100 R13**	89.9	***S. aureus* 100 R13**	89.0	***A. baumannii* 100 R13**	88.0
***E. coli* 100 R14**	90.1	***S. aureus* 100 R14**	89.0	***A. baumannii* 100 R14**	88.9
***E. coli* 100 R15**	90.2	***S. aureus* 100 R15**	88.3	***A. baumannii* 100 R15**	90.0
***E. coli* 100 R16**	90.9	***S. aureus* 100 R16**	90.0	***A. baumannii* 100 R16**	91.4
***E. coli* 200 R1**	89.7	***S. aureus* 200 R1**	82.1	***A. baumannii* 200 R1**	82.1
***E. coli* 200 R2**	90.0	***S. aureus* 200 R2**	85.3	***A. baumannii* 200 R2**	84.3
***E. coli* 200 R3**	83.2	***S. aureus* 200 R3**	85.3	***A. baumannii* 200 R3**	85.3
***E. coli* 200 R4**	85.9	***S. aureus* 200 R4**	87.0	***A. baumannii* 200 R4**	86.4
***E. coli* 200 R5**	87.6	***S. aureus* 200 R5**	89.0	***A. baumannii* 200 R5**	86.3
***E. coli* 200 R6**	88.3	***S. aureus* 200 R6**	89.3	***A. baumannii* 200 R6**	87.4
***E. coli* 200 R7**	90.3	***S. aureus* 200 R7**	89.0	***A. baumannii* 200 R7**	87.3
***E. coli* 200 R8**	92.2	***S. aureus* 200 R8**	90.0	***A. baumannii* 200 R8**	87.4
***E. coli* 200 R9**	93.4	***S. aureus* 200 R9**	91.2	***A. baumannii* 200 R9**	88.0
***E. coli* 200 R10**	94.3	***S. aureus* 200 R10**	91.4	***A. baumannii* 200 R10**	88.0
***E. coli* 200 R11**	96.9	***S. aureus* 200 R11**	93.0	***A. baumannii* 200 R11**	90.1
***E. coli* 200 R12**	90.2	***S. aureus* 200 R12**	96.5	***A. baumannii* 200 R12**	91.2
***E. coli* 200 R13**	90.3	***S. aureus* 200 R13**	88.0	***A. baumannii* 200 R13**	86.3
***E. coli* 200 R14**	90.5	***S. aureus* 200 R14**	89.3	***A. baumannii* 200 R14**	87.0
***E. coli* 200 R15**	89.5	***S. aureus* 200 R15**	88.4	***A. baumannii* 200 R15**	87.0

***E. coli* 200 R16**	89.8	***S. aureus* 200 R16**	89.0	***A. baumannii* 200 R16**	87.2
***E. coli* 400 R1**	89.2	***S. aureus* 400 R1**	90.2	***A. baumannii* 400 R1**	80.0
***E. coli* 400 R2**	86.3	***S. aureus* 400 R2**	92.3	***A. baumannii* 400 R2**	82.3
***E. coli* 400 R3**	87.0	***S. aureus* 400 R3**	93.2	***A. baumannii* 400 R3**	83.2
***E. coli* 400 R4**	88.0	***S. aureus* 400 R4**	96.0	***A. baumannii* 400 R4**	84.3
***E. coli* 400 R5**	88.0	***S. aureus* 400 R5**	86.5	***A. baumannii* 400 R5**	87.4
***E. coli* 400 R6**	81.0	***S. aureus* 400 R6**	87.4	***A. baumannii* 400 R6**	88.3
***E. coli* 400 R7**	84.1	***S. aureus* 400 R7**	87.0	***A. baumannii* 400 R7**	90.0
***E. coli* 400 R8**	85.1	***S. aureus* 400 R8**	88.2	***A. baumannii* 400 R8**	91.2
***E. coli* 400 R9**	91.3	***S. aureus* 400 R9**	80.1	***A. baumannii* 400 R9**	84.3
***E. coli* 400 R10**	91.8	***S. aureus* 400 R10**	83.2	***A. baumannii* 400 R10**	85.3
***E. coli* 400 R11**	93.5	***S. aureus* 400 R11**	83.9	***A. baumannii* 400 R11**	85.4
***E. coli* 400 R12**	93.5	***S. aureus* 400 R12**	84.3	***A. baumannii* 400 R12**	86.3
***E. coli* 400 R13**	97.5	***S. aureus* 400 R13**	87.5	***A. baumannii* 400 R13**	86.0
***E. coli* 400 R14**	89.0	***S. aureus* 400 R14**	89.0	***A. baumannii* 400 R14**	87.2
***E. coli* 400 R15**	90.0	***S. aureus* 400 R15**	89.1	***A. baumannii* 400 R15**	87.3
***E. coli* 400 R16**	90.2	***S. aureus* 400 R16**	90.2	***A. baumannii* 400 R16**	87.2
***E. coli* 600 R1**	89.0	***S. aureus* 600 R1**	88.0	***A. baumannii* 600 R1**	88.0
***E. coli* 600 R2**	89.0	***S. aureus* 600 R2**	89.1	***A. baumannii* 600 R2**	89.0
***E. coli* 600 R3**	90.0	***S. aureus* 600 R3**	89.1	***A. baumannii* 600 R3**	90.2
***E. coli* 600 R4**	91.2	***S. aureus* 600 R4**	89.3	***A. baumannii* 600 R4**	91.4
***E. coli* 600 R5**	80.3	***S. aureus* 600 R5**	91.2	***A. baumannii* 600 R5**	84.3
***E. coli* 600 R6**	84.5	***S. aureus* 600 R6**	92.3	***A. baumannii* 600 R6**	85.3
***E. coli* 600 R7**	85.9	***S. aureus* 600 R7**	92.6	***A. baumannii* 600 R7**	85.3
***E. coli* 600 R8**	87.0	***S. aureus* 600 R8**	96.2	***A. baumannii* 600 R8**	86.3
***E. coli* 600 R9**	86.8	***S. aureus* 600 R9**	80.2	***A. baumannii* 600 R9**	85.3
***E. coli* 600 R10**	87.5	***S. aureus* 600 R10**	82.4	***A. baumannii* 600 R10**	86.4
***E. coli* 600 R11**	87.5	***S. aureus* 600 R11**	84.5	***A. baumannii* 600 R11**	86.3
***E. coli* 600 R12**	90.0	***S. aureus* 600 R12**	85.0	***A. baumannii* 600 R12**	87.5
***E. coli* 600 R13**	91.5	***S. aureus* 600 R13**	86.2	***A. baumannii* 600 R13**	80.3
***E. coli* 600 R14**	93.9	***S. aureus* 600 R14**	87.3	***A. baumannii* 600 R14**	82.3
***E. coli* 600 R15**	94.3	***S. aureus* 600 R15**	86.5	***A. baumannii* 600 R15**	83.4
***E. coli* 600 R16**	96.9	***S. aureus* 600 R16**	88.0	***A. baumannii* 600 R16**	84.3
***E. coli* 800 R1**	87.6	***S. aureus* 800 R1**	88.0	***A. baumannii* 800 R1**	84.5
***E. coli* 800 R2**	90.3	***S. aureus* 800 R2**	88.0	***A. baumannii* 800 R2**	87.4

***E. coli* 800 R3**	90.0	***S. aureus* 800 R3**	89.0	***A. baumannii* 800 R3**	87.0
***E. coli* 800 R4**	91.2	***S. aureus* 800 R4**	90.1	***A. baumannii* 800 R4**	87.0
***E. coli* 800 R5**	91.2	***S. aureus* 800 R5**	84.2	***A. baumannii* 800 R5**	88.0
***E. coli* 800 R6**	93.4	***S. aureus* 800 R6**	85.3	***A. baumannii* 800 R6**	89.3
***E. coli* 800 R7**	93.5	***S. aureus* 800 R7**	85.3	***A. baumannii* 800 R7**	90.5
***E. coli* 800 R8**	96.9	***S. aureus* 800 R8**	87.3	***A. baumannii* 800 R8**	92.3
***E. coli* 800 R9**	85.0	***S. aureus* 800 R9**	90.5	***A. baumannii* 800 R9**	79.0
***E. coli* 800 R10**	86.5	***S. aureus* 800 R10**	92.3	***A. baumannii* 800 R10**	80.2
***E. coli* 800 R11**	85.2	***S. aureus* 800 R11**	93.2	***A. baumannii* 800 R11**	80.5
***E. coli* 800 R12**	88.0	***S. aureus* 800 R12**	96.3	***A. baumannii* 800 R12**	82.5
***E. coli* 800 R13**	79.9	***S. aureus* 800 R13**	79.0	***A. baumannii* 800 R13**	83.5
***E. coli* 800 R14**	82.3	***S. aureus* 800 R14**	81.2	***A. baumannii* 800 R14**	83.5
***E. coli* 800 R15**	83.3	***S. aureus* 800 R15**	82.2	***A. baumannii* 800 R15**	83.4
***E. coli* 800 R16**	84.4	***S. aureus* 800 R16**	82.3	***A. baumannii* 800 R16**	85.6
***E. coli* 1000 R1**	80.2	***S. aureus* 1000 R1**	74.9	***A. baumannii* 1000 R1**	80.4
***E. coli* 1000 R2**	81.1	***S. aureus* 1000 R2**	77.3	***A. baumannii* 1000 R2**	80.2
***E. coli* 1000 R3**	81.3	***S. aureus* 1000 R3**	78.3	***A. baumannii* 1000 R3**	80.9
***E. coli* 1000 R4**	82.3	***S. aureus* 1000 R4**	79.5	***A. baumannii* 1000 R4**	82.4
***E. coli* 1000 R5**	87.1	***S. aureus* 1000 R5**	86.3	***A. baumannii* 1000 R5**	75.8
***E. coli* 1000 R6**	88.3	***S. aureus* 1000 R6**	88.3	***A. baumannii* 1000 R6**	77.2
***E. coli* 1000 R7**	89.3	***S. aureus* 1000 R7**	88.5	***A. baumannii* 1000 R7**	78.3
***E. coli* 1000 R8**	91.4	***S. aureus* 1000 R8**	91.9	***A. baumannii* 1000 R8**	79.3
***E. coli* 1000 R9**	83.0	***S. aureus* 1000 R9**	84.0	***A. baumannii* 1000 R9**	84.5
***E. coli* 1000 R10**	85.2	***S. aureus* 1000 R10**	85.0	***A. baumannii* 1000 R10**	86.0
***E. coli* 1000 R11**	85.0	***S. aureus* 1000 R11**	85.0	***A. baumannii* 1000 R11**	87.2
***E. coli* 1000 R12**	85.5	***S. aureus* 1000 R12**	86.3	***A. baumannii* 1000 R12**	89.0
***E. coli* 1000 R13**	74.9	***S. aureus* 1000 R13**	80.4	***A. baumannii* 1000 R13**	81.5
***E. coli* 1000 R14**	77.3	***S. aureus* 1000 R14**	80.5	***A. baumannii* 1000 R14**	84.2
***E. coli* 1000 R15**	78.4	***S. aureus* 1000 R15**	81.4	***A. baumannii* 1000 R15**	83.4
***E. coli* 1000 R16**	78.6	***S. aureus* 1000 R16**	83.0	***A. baumannii* 1000 R16**	83.5

***E. coli* 1100 R1**	75.5	***S. aureus* 1100 R1**	75.7	***A. baumannii* 1100 R1**	74.8
***E. coli* 1100 R2**	76.8	***S. aureus* 1100 R2**	75.7	***A. baumannii* 1100 R2**	74.9
***E. coli* 1100 R3**	76.5	***S. aureus* 1100 R3**	76.3	***A. baumannii* 1100 R3**	75.9
***E. coli* 1100 R4**	75.8	***S. aureus* 1100 R4**	74.9	***A. baumannii* 1100 R4**	74.5
***E. coli* 1100 R5**	75.2	***S. aureus* 1100 R5**	76.4	***A. baumannii* 1100 R5**	73.9
***E. coli* 1100 R6**	75.4	***S. aureus* 1100 R6**	76.8	***A. baumannii* 1100 R6**	75.0
***E. coli* 1100 R7**	75.1	***S. aureus* 1100 R7**	74.8	***A. baumannii* 1100 R7**	75.0
***E. coli* 1100 R8**	75.3	***S. aureus* 1100 R8**	75.6	***A. baumannii* 1100 R8**	76.8
***E. coli* 1200 R1**	69.5	***S. aureus* 1200 R1**	69.1	***A. baumannii* 1200 R1**	67.5
***E. coli* 1200 R2**	69.2	***S. aureus* 1200 R2**	69.8	***A. baumannii* 1200 R2**	68.3
***E. coli* 1200 R3**	68.3	***S. aureus* 1200 R3**	69.1	***A. baumannii* 1200 R3**	68.4
***E. coli* 1200 R4**	69.5	***S. aureus* 1200 R4**	68.4	***A. baumannii* 1200 R4**	68.3
***E. coli* 1200 R5**	69.4	***S. aureus* 1200 R5**	70.8	***A. baumannii* 1200 R5**	68.4
***E. coli* 1200 R6**	68.3	***S. aureus* 1200 R6**	70.0	***A. baumannii* 1200 R6**	69.2
***E. coli* 1200 R7**	69.2	***S. aureus* 1200 R7**	68.3	***A. baumannii* 1200 R7**	69.2
***E. coli* 1200 R8**	69.1	***S. aureus* 1200 R8**	69.1	***A. baumannii* 1200 R8**	68.4

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