AmeboGenesis Raw Unprocessed Lysate (AG-RUL) as a Platform for Rapid, Broad-Range Detection and Removal of Pathogenic Bacteria and Associated Endotoxins from Complex Matrix such as Human Serum

Authors: D. Kilbank, W. Tutak, G. Kaufman

Abstract

Antibiotics have been central to infectious disease management for nearly a century, yet rising **antimicrobial resistance** and persistent mortality associated with inflammatory conditions such as **sepsis** highlight limitations of therapeutic strategies focused primarily on microbial eradication. Increasing evidence indicates that many severe outcomes of infection arise from host immune responses triggered by **microbial molecular signals, including endotoxins, beta glucans and other pathogen-associated molecular patterns.** The **AmeboGenesis™** platform explores a new approach to detecting and managing these signals by leveraging the extraordinary sensitivity of **amebocytes derived from ancient innate immune systems.** Using the **Mygotic Process™,** scalable production of amebocytes enables technologies that extend beyond traditional Limulus Amebocyte Lysate testing. This paper discusses **AG-RUL™**, a platform developed for **rapid detection and removal of endotoxins, beta glucans and related inflammatory signals** from human biological fluids, including **serum and cerebrospinal fluids**, with additional applications in infection diagnostics, critical care monitoring, and sepsis management.

Infection → Endotoxin Release → Inflammatory Cascade - AG-RUL™ = Rapid Detection & Removal

Keywords: AG-RUL, AmeboGenesis, Mygotic Process, EndotoxinDetection, Sepsis, InnateImmuneBiology, MolecularInfectionControl, AntimicrobialResistance, Amebocytes, Infection

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Preface

Rethinking Infection - A New Biological Strategy

For nearly a century, antibiotics have been the cornerstone of infectious disease treatment. Their discovery transformed modern medicine, enabling complex surgery, reducing mortality

from bacterial infections, and dramatically improving life expectancy worldwide. Yet the effectiveness of this model is increasingly under pressure.

Antimicrobial resistance is now widely recognized as one of the most serious threats to global health. Public health authorities warn that the continued rise of resistant pathogens could lead to a "post-antibiotic era," in which common infections once again become difficult to treat. However, resistance may not be the only challenge confronting modern infection management.

Many of the most dangerous consequences of infection arise not solely from the presence of microorganisms, but from the molecular signals they release. Endotoxins and other pathogen-associated molecules can trigger powerful inflammatory responses in the human body, contributing to conditions such as sepsis, even after microbes themselves have been reduced or eliminated. What if the future of infection control is not about killing microbes, but managing what actually makes us sick?

This perspective suggests that infectious disease may be understood not only as a microbial problem, but also as a problem of **molecular signaling and immune activation.** Advances in biotechnology may now allow these signals to be detected and potentially managed with unprecedented sensitivity.

The AmeboGenesis™ **AG-RUL™** program is built upon the **Mygotic Process™**, a biological platform capable of generating large quantities of amebocytes derived from ancient innate immune systems. This could also extend their capabilities beyond traditional testing applications.These cells possess extraordinary sensitivity to microbial components, a property that historically enabled the development of the **Limulus Amebocyte Lysate (LAL)** assay, the gold standard for endotoxin detection in pharmaceuticals and medical devices.

Emerging platforms such as the AmeboGenesis **AG-LAL™** and **AG-RUL™** programs illustrate how this biology can support next-generation endotoxin & beta glucan detection as well as new approaches to identifying and potentially removing pathogenic molecular signals from serum, cerebrospinal, and other human fluids.

If realized, these technologies could expand the way infection is detected, monitored, and ultimately managed. This white paper explores the scientific foundations and data behind this platform and considers how advances in innate immune biology may contribute to a broader rethinking of infectious disease strategy.

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Introduction

Modern approaches to detecting, eliminating, and removing microbes (particularly Gram-negative pathogens) from human blood and components (e.g., whole blood, plasma, serum, and other products), through cerebrospinal fluid, and other specific body fluids required for deeper clinical evaluation have advanced rapidly through high-resolution sequencing[1], digital PCR[2], molecular diagnostics[3], spectroscopy[4] and endotoxin-specific assays (LAL/Factor-C)[5-7], yet major challenges remain. Performance of these detection platforms is affected by the presence of various interfering chemicals, host-derived inhibitors, cells, and microbial debris, while traditional culture methods are slow and insensitive[8-10]. Microbial elimination strategies have relied on application of broad-spectrum antibiotics and chemical inactivation, which can effectively kill bacteria but suffer from significant drawbacks including, systemic side effects, long treatment times, and inflated cost[11-13]. These limitations have driven a shift toward more refined physical and affinity-based removal technologies, including nano-filtration[14], dialysis[15], magnetic bio separation[16], and adsorption[17], which offer the advantage of extracting whole pathogens and toxic byproducts (e.g., endotoxins) rather than leaving inflammatory residues behind. However, these systems still struggle with instrument fouling, limited specificity, and lack of standardized workflows[18-20]. Although LAL kits are sensitive, their use in serum is limited because blood proteins and lipids can mask endotoxin and block enzyme activation, producing unreliable results. The FDA has not accepted LAL for detecting systemic endotoxemia[5], and pretreatment steps such as heating denaturation of interfering proteins, dilution with pyrogen-free water, and heparin treatment only partially reduce interference but do not eliminate variability[5, 21-23]. These limitations have driven interest in alternatives like recombinants (cascade factors or factor C) and neutrophil-based endotoxin activity assay (EAA). Although the recombinants avoid the need for horseshoe crab (HSC) blood, they remain a synthetic analogue of the natural enzyme cascade and are not universally endorsed as a compendial method by regulators such as USP <85>, requiring extensive validation for FDA-regulated use[6,7,24-26]. EAA measures leukocyte activation rather than the actual endotoxin concentration, making results sensitive to patient-specific factors like immune suppression and limiting its ability to detect low-grade metabolic endotoxemia[27,28]. Therefore, current solutions are not efficient because they address detection,

elimination, or removal separately rather than as an integrated process in a complex matrix such as serum. Our sustainable AG-RUL is proposed as an alternative to traditional LAL since it incorporates the full native LAL cascade without relying on horseshoe crabs as a source, instead using embryonic stem cell lines (AG-Ameboblasts) derived from HSC somatic cells via the Mygotic Process™ differentiated into native amebocyte-like cells, enabling direct endotoxin detection. The critical next step is the development of a unified, domain-specific, real-time platform that combines high affinity detection and effective removal of pathogens and their toxic byproducts from complex clinical samples such as patients' blood and serum with greater precision, safety, and speed.

Limitations and challenges of antibiotic use

Antibiotics are essential for treating bacterial infections but have significant limitations that impact both clinical outcomes and public health[29-32]. Their overuse drives the emergence and spreads of antimicrobial-resistant strains that indiscriminately disrupt the normal human microbiome, which in turn increases the risk of secondary infections and long-term health consequences[33-36]. Antibiotics kill bacteria without removing endotoxins, which can cause adverse inflammatory reactions (e.g., bacterial shedding) and organ toxicity[5,28,37-39]. Antibiotics are ineffective against viral and fungal infections, rely on strict patient adherence, and contribute to environmental resistance through residual contamination[40-43]. The slow development of new antibiotic classes and the growing prevalence of multidrug-resistant pathogens (superbugs)[30,31,44,45] underscore the need for appropriate use, robust stewardship programs, and the pursuit of alternative therapies that target pathogens more specifically and safely.

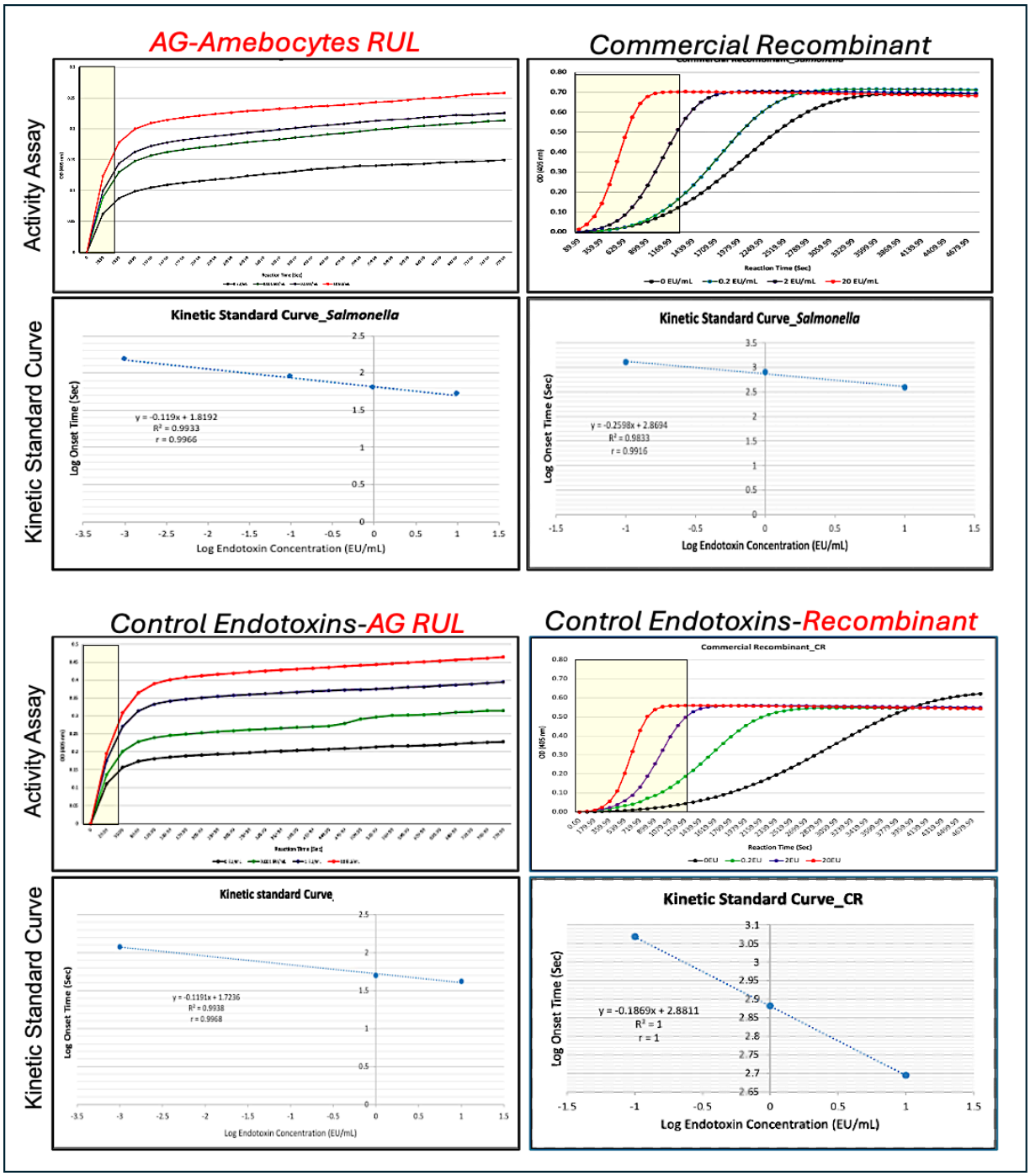
Advantages of non-antibiotic pathogen removal strategies

Non-antibiotic pathogen removal strategies provide significant advantages by physically eliminating bacteria and their toxins rather than relying solely on bactericidal mechanisms of action, thereby avoiding selective pressure for resistance and preserving sensitive clinical materials such as blood, therapeutic cells, and tissues. These various removal approaches include bacteriophages[46], antimicrobial peptides[47], CRISPR-Cas systems[48], immune-based therapies[49], physical filtration[50], and affinity capture[51] offer high specificity, preserving beneficial microbiota while effectively targeting antibiotic-resistant, dormant, biofilm-associated, or intracellular pathogens. By removing whole bacteria along with endotoxins, they reduce inflammatory risks, improve diagnostic accuracy, and can be integrated into automated workflows[37,50-52]. Acting through non-traditional mechanisms rather than growth inhibition minimizes resistance development, reduces systemic toxicity, avoids drug interactions, and allows rapid pathogen clearance[47,48,53,54], making them particularly valuable for high-risk applications

such as sepsis management, transfusion safety, and extracorporeal pathogen removal in clinical and advanced therapeutic settings[18,20,55,56].

Operational barriers of current bacterial-removal approaches in clinical samples

Pathogen removal approach offers a significant advantage over detection-only techniques and inactivation-based approaches that inactivate bacteria without clearing their inflammatory components by physically extracting microbes and their toxic byproducts from blood and therefore improving quality of the patient treatment and reducing reliance on antibiotics. Current physical removal methods, including microfluidics, affinity capture, hemoperfusion, and bioinspired platforms, demonstrate clinical potential but face major limitations when operating in complex matrices (e.g., blood and its components) including slow binding kinetics, non-specific interactions, and a very limited ability to detect endotoxins[18,20,51,57,58]. While some devices remove endotoxin or inflammatory mediators, none of them efficiently eliminate both bacteria and their toxic byproducts while providing broad pathogen detection. Integrating broad-spectrum, real-time detection into a pathogen removal platform could transform these technologies into diagnostic-therapeutic systems, allowing simultaneous bacterial capture, quantification, and species-level identification, thereby accelerating targeted therapy, guiding key antimicrobial therapy decisions (e.g., agent selection, dosage, and duration), and providing immediate feedback on treatment efficacy. Overall, existing methods highlight the need for reliable, scalable filtration systems capable of safely removing pathogens while providing fast quantifiable feedback on bacterial-removal efficacy.



Development of a novel platform based on sustainable AG-RUL to rapidly detect a wide range of bacterial pathogens and their endotoxins prior to their removal.

1. Comparison of LAL activity-assay alternatives and their related kinetic standard curves to *Salmonella* endotoxin

2. Pathogen-derived endotoxins detected by AG-RUL

Bacterial Strain Endotoxin	Pathogenic Effect
Salmonella enterica	gastroenteritis, septic shock
Klebsiella pneumoniae	pneumoniae, sepsis, lung diseases, tissue damages
Pseudomonas aeruginosa	pneumoniae, sepsis, skin lesions, tissue destruction
Escherichia coli O55:B5	septic shock, significant fever
Escherichia coli O157:H7	abdominal cramps and diarrhea, hemolytic anemia, kidney failure
Escherichia coli O111:B4	fever, bloody diarrhea, hemolytic anemia

3. AG-RUL system model workflow for detection-removal

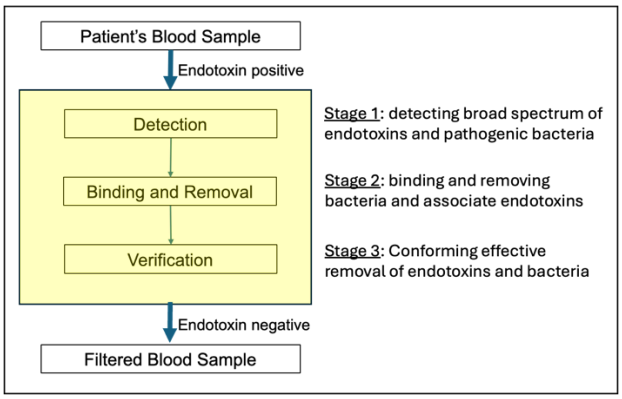


Fig. 1. Comparison of activity assays (optical density (OD) at 405 nm vs. reaction time (seconds)) and their corresponding kinetic standard curves (log onset time (Sec) vs. log endotoxin concentration (EU/mL) for *Salmonella enterica* endotoxin as an alternative to the traditional LAL: AG-Amebocytes RUL (left top panel) and commercial recombinant (right top panel). The control endotoxin detected by the AG-RUL (left bottom panel) and commercial recombinant RUL display similar activity curves (bottom right panel). Fig. 2. Table summarizes bacterial pathogens and their associated clinical effects, as tested and identified by the AG-RUL at a range of concentrations. Fig. 3. Schematic diagram illustrates the design of the sustainable AG-Technology workflow for pathogen/endotoxin identification, removal, and verification to obtain a cleared sample.

AG-RUL yielded a shorter endotoxin readout time than commercial alternatives (curves enclosed in the yellow boxes), improving assay efficiency and increasing its throughput (Fig. 1). AG-RUL produced kinetic standard curves with linearity that meet industrial performance criteria ($r \geq 0.98$) when evaluated across a range of endotoxin concentrations from the pathogens listed in Fig. 2 and the control endotoxin as shown in Fig. 1. The assays showed higher sensitivity and detected a broader dynamic range of endotoxin concentrations, with standard curves extending to levels as low as 0.001 EU/mL (Fig. 3: kinetic standard curves). Our sustainable AG-RUL system produced from amebocytes (differentiated horseshoe crab embryonic stem cells generated through the Mygotic Process™)[135] demonstrated the ability to detect a wide range of endotoxins (10 – 0.001 EU/mL) associated with various bacterial pathogens, as described in Figs. 1 and 2. These results demonstrate the sensitivity and efficiency of our platform and support the development of an integrated system capable of detecting, removing and confirming clearance of endotoxins and their associated bacteria from infected patient blood and its components as illustrated in Fig. 3.

The essential role of human serum in clinical and industrial applications

Human serum and serum-derived products play essential roles in both clinical care and industrial biotechnology. Antiserum provides immediate passive immunity through pre-formed antibodies[59], including antivenoms[60], antitoxins[61] for life-threatening infections[63], and convalescent serum for several viral infections (e.g., Ebola and COVID-19)[64]. Serum is the preferred diagnostic matrix because it lacks clotting factors (fibrinogen-free) that interfere with testing, supporting blood-bank crossmatching, metabolic and organ-function testing, tumor-marker analysis, and HLA typing[65-69]. Albumin, serum's major protein, is routinely infused for blood volume expansion in burns, hemorrhage, surgery, and ascites management in liver failure[70-73]. Autologous serum is used in eye drops for severe dry eye and corneal injury[74,75], while cleaned "platelet-rich" serum is used to accelerate the closing of chronic ulcers that would not heal in standard procedures[76,77]. In industry, human serum is a key raw material for biomanufacturing and regenerative medicine. AB serum (free of anti-A and anti-B antibodies) supports sensitive cell lines[78,79], Off-the-clot (OTC) serum provides growth factors for primary cells in cell therapy, injectable biologics where anticoagulants would be problematic[80,81], and the development of in vitro diagnostic assays[65]. Plasma-derived serum, generated by clotting anticoagulated donor plasma, supports large-scale vaccine development[78,79,82]. Human serum also supplements therapeutic cell cultures to avoid immunogenicity associated with animal sera[79]. Albumin stabilizes vaccines and biologics, and serum matrices serve as negative control in diagnostic kits[83,84]. Albumin functions as a drug carrier protein for targeted drug delivery[85,86]. Since microbial contamination can compromise safety and performance, rigorous pathogen detection and purification are essential to maintain serum quality across all applications.

Challenges in bacterial and endotoxin detection and removal from human serum

Detecting bacteria and endotoxins in human serum is extremely challenging. It is one of the most complex biological matrices, containing abundant proteins, lipids, and antibodies that interfere with detection. Albumin and immunoglobulins can coat bacteria or bind endotoxins, masking targets and preventing access by molecular primers or LAL reagents[6,87,88]. Serum nucleases can degrade microbial DNA, and proteases can break down bacterial proteins[89,90]. Treatment with anticoagulants such as heparin or EDTA can inhibit enzymatic steps in molecular assays[91-93]. In clinical cases like sepsis, bacterial or endotoxin levels are often very low, while background serum components are high, further reducing sensitivity[94-96]. Culture-based methods require 24-72 hours, which is too slow for effective critical care[97,98]. Serum factors may activate or inhibit cascade-based detection kits, forcing dilution or heat treatment[99,100]. Molecular assays must overcome the substantial background of host (human) DNA before bacterial DNA can be analyzed[101], and mass spectrometry requires sample-preparation steps to isolate

microbial material from serum[102]. To overcome these barriers, detection must shift toward systems that mimic the sensitivity and selectivity of native amebocytes, able to sense and trace endotoxins in dense serum matrices rapidly, reliably, and without extensive sample manipulations.

Human serum is filtered to achieve sterility, remove bacterial and endotoxins, and protect patients. Common approaches include 0.22-micron PES or PVDF membrane filters, affinity filters that bind microbes through biochemical interactions, nanofilters with 20-30 nm pores, and charged membranes that capture negatively charged pathogens[103-107]. Filtration, however, has significant drawbacks: viscous serum clogs membranes, small bacteria may pass through, and filters can remove essential proteins, lipids, hormones, and vitamins. Extracellular vesicles may rupture under pressure, and trapped bacteria can continue releasing endotoxins[58,107,108-112]. Adsorbent beads saturate quickly, and charged filters may remove needed molecules[113-115]. Alternative decontamination methods also introduce challenges: heat inactivation damage and denature essential proteins and growth factors[116,117], gamma irradiation alters cytokines and generates free radicals[118-120], UV-C is ineffective in turbid samples and degrades serum components, chemical agents leave toxic residues[121-125], and high-speed centrifugation rarely removes all microbial contaminants[58,126]. Additional endotoxin-removal tools such as polymyxin-based adsorbents bind poorly in protein-rich serum and lose efficiency as columns saturate[20,127,128]. More advanced methods including ion exchange chromatography and detergent-based phase separation are highly sensitive to pH and salt concentration and can denature proteins and leave residual reagents[129-132]. Some industrial processes use chemical neutralization, but chemical residues and extreme pH conditions can damage serum components[132-134]. There is a need to design a detection and removal system tailored to the specific conditions of serum that does not rely on filtration membrane or alterations to environmental or serum conditions. Such a system would employ specific binding mechanisms that naturally and selectively target bacteria without becoming saturated, while preserving serum integrity.

AG-RUL enables endotoxin detection in human serum at concentrations as low as 0.01 EU/mL.

4. AG-RUL recovers 0.01 EU/ML of spiked endotoxin from concentrated and diluted human serum sample

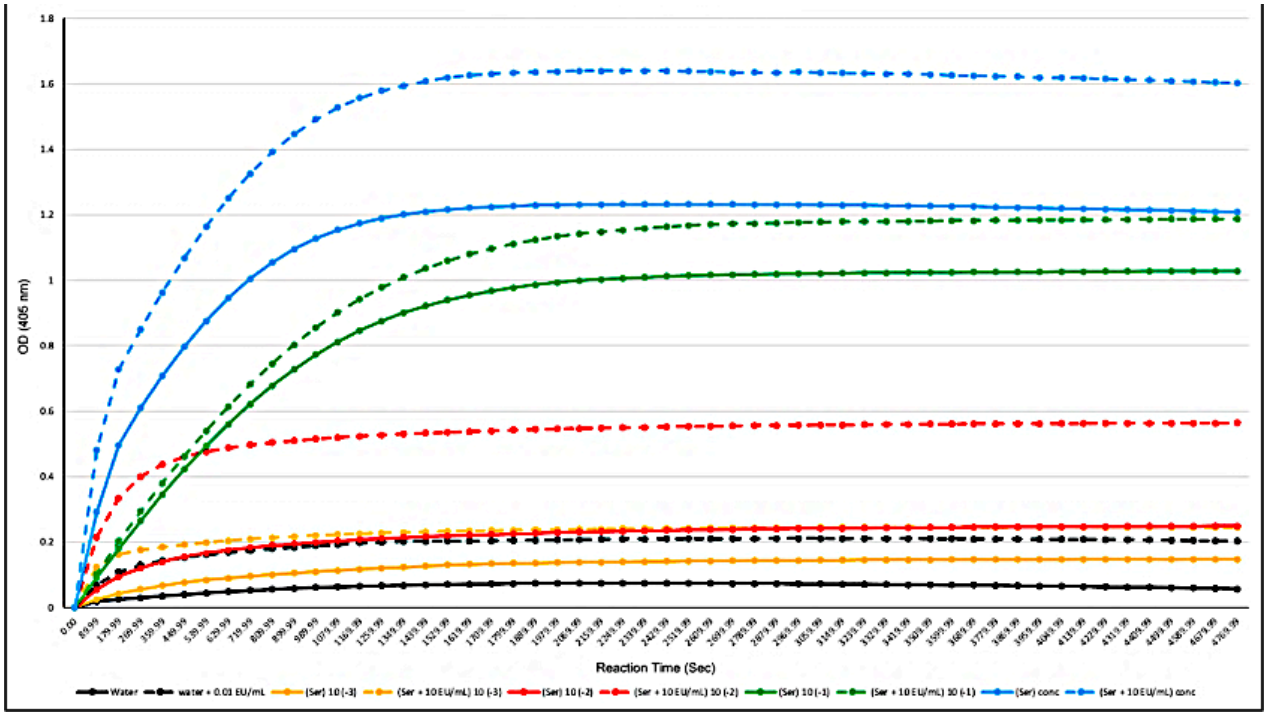


Fig. 4. Activity assay of AG-RUL (optical density (OD) at 405 nm vs. reaction time (seconds)) detecting commercial endotoxin at 0.01 EU/mL in human serum, tested undiluted (blue curve) and in serial dilutions of 10^{-1} (green), 10^{-2} (red), and 10^{-3} (yellow), along with a no-endotoxin control (black). Assays containing the endotoxin (dashed line) were compared with those without the endotoxin (solid line). Fig. 5. Activity assays of AG-RUL on serum samples from three donors of different genders and ages: young male (left), an elderly adult (center), and a young female (right). Same color code as the previous figure.

AG-RUL recovered endotoxin at a minimum concentration of 0.01 EU/mL, consistent with sensitivity levels typically applied in industry for samples with strict bacterial-load requirements such as cerebrospinal fluid. The ability to detect low endotoxin levels in the blood (approximately 0.5 EU/mL) enables early identification of bacteria before they propagate, cause sepsis, and spread to other tissues. No dilution was required to detect the endotoxin directly in serum, although serial dilutions produced comparable results. The most effective recovery occurred in concentrated serum and in 10^{-1} and 10^{-2} dilutions (Fig. 4). Assay reproducibility was demonstrated across three donors of different ages and genders, indicating that AG-RUL endotoxin-detection capability is applicable without patient-specific age or gender limitations (Fig. 5).

Unlimited LAL source for detection and elimination of pathogenic bacteria and their associated endotoxins in human serum

A continuous supply of Limulus amebocyte lysate (LAL) or factors cascade (Factor C, Factor B, and Pro-clotting Enzymes)[135] provides sensitive, specific, and consistent detection of endotoxins and their associated bacterial pathogens, overcoming batch variability and offering a stable, renewable reagent source for complex clinical matrices such as blood and its derived products (serum). By enabling rapid identification of Gram-negative pathogens independent of bacterial viability, which is whether the bacteria are alive or dead, allowing the detection of bacterial components within complex matrix components without the need to heat, treat with heparin, or even dilute when analyzed directly from concentrated serum. These systems allow real-time or high-frequency monitoring, integration into automated or continuous-flow platforms, and uninterrupted supply in high-throughput operations. Continuous availability of purified Factor C supports sustained and reliable surveillance in applications such as blood filtration in extracorporeal circuits, and tissue perfusion, while reducing false positives and preserving host cells. When combined with physical removal of endotoxins, LAL-based systems not only detect but also help mitigate endotoxin and bacterial load, enhancing patient safety, protecting therapeutic products, and maintaining compliance with stringent purity standards without introducing antibiotics or promoting resistance.

Enhancing safety and efficacy with rapid, broad range endotoxin detection using LAL testing

Rapid LAL testing with a wide dynamic range of endotoxin detection enhances the ability of clinical and industrial settings to identify and control Gram-negative contamination in blood derived products such as serum, and other sensitive materials. By detecting endotoxin quickly across wide range of concentrations, these assays will provide early endotoxin warning before culture detects growth and may yield expedited results than slower-processing molecular methods, enabling earlier clinical intervention in sepsis and more reliable quality control in blood products, cell therapies, and tissue preparations. Broad coverage allows laboratories to monitor low level and early stage emerging or high load contaminations without adjusting assays or improving workflow efficiency. Because LAL via Factor C activation detects lipopolysaccharide independent of bacterial viability, it captures live, damaged, or lysed bacteria that conventional methods may miss, facilitating early risk assessment and classification, improved patient outcomes, and adaptive industrial manufacturing with reduced batch loss. When integrated with traditional diagnostics and automated systems, rapid LAL testing supports measurements across diverse concentrations within its dynamic range, transforming endotoxin detection from a downstream check into a proactive, real-time surveillance tool that enhances safety, responsiveness, and decision-making in both healthcare and bioprocessing applications.

Conclusion

These results demonstrate a sustainable, sensitive AG-RUL platform capable of rapidly detecting toxins associated with a variety of common bacterial pathogens across wide concentration ranges, directly within complex matrices such as human serum. By pairing this detection capability with a complementary mechanism for selectively removing contaminants, the system lays out the groundwork for a fully integrated detect-and-clear workflow. Such an approach would not only improve sample clarity by removing pathogens and endotoxins and providing a cleaner, pathogen-reduced sample, but also support safer and more reliable downstream clinical and manufacturing processes that rely on complex matrices such as human serum. As development continues, the envisioned unified platform has the potential to redefine quality by enabling real-time monitoring, streamlined and controlled purification, and greater confidence in the integrity of complex biological and clinical samples such as serum.

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