Sepsis is a life-threatening dysfunction of organ systems caused by a dysregulated immune system because of an infectious process. It remains one of the leading causes of hospital mortality and of hospital readmissions in the United States. Mortality from sepsis increases with each hour of delayed treatment, therefore, diagnostic devices that can reduce the time from the onset of a patient’s infection to the delivery of appropriate therapy are urgently needed. Likewise, tools that are capable of high-frequency testing of clinically relevant biomarkers are required to study disease progression. Electrochemical biosensors offer important advantages such as high sensitivity, fast response, miniaturization, and low cost that can be adapted to clinical needs. In this review paper, we discuss the current state, limitations, and future directions of electrochemical-based point-of-care detection platforms that contribute to the diagnosis and monitoring of sepsis.

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**Keywords**

**Introduction**
Based on the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [1], sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. This definition underscores the relevance of both collecting pathogen information and integrating this information with host response monitoring [2]. Likewise, identifying patients early in their infectious process remains problematic. It has been recommended that adult patients with suspected infection can be screened as positive for sepsis if they score 2 or more points on the quickSOFA score: respiratory rate $>22$/min, altered mental state, or systolic blood pressure $>100$ mm Hg [1]. This criterion is simple enough to use in the outpatient setting, emergency department and in-hospital. However, this criterion is neither sensitive nor specific enough to be a standalone identifier of the septic patient.

If an infection is suspected in hospital environments, broad-spectrum antibiotics are typically immediately prescribed, followed by a test for the presence of bacteria (bacterial culture and growth) and a test for pathogen identification (PCR) [2]. Sepsis can be caused by infection from bacterial, viral, and fungal pathogens including but not limited to *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), herpes simplex virus, influenza viruses, and *Candida albicans* fungus [3–5] (Table 1). Unfortunately, blood culture analysis takes 1–5 days to resolve (5 days for confirmed negative) and yields false negative and false positive results [6]. Likewise, both the blood culture and the subsequent PCR analysis require specialized laboratory facilities and cannot be performed at the point-of-care.
Regarding the monitoring of the host response, while bloodborne biomarkers (circulating proteins, cell-surface proteins, microRNA) are commonly used as they are associated with specific sepsis stages and can detect sepsis before the appearance of physical symptoms [7], no single biomarker is specific enough to diagnose or stratify sepsis [2,8]. Furthermore, biomarker concentrations constantly vary, highlighting the need for high-frequency and cost-effective testing of a large number of biomarkers.

Electrical- and electrochemical-based devices with multiplexing abilities hold significant potential for providing near real-time monitoring of pathogens and sepsis biomarkers in point of care (POC) settings, such as emergency rooms, doctor’s offices, and ambulance services, where septic patients first encounter healthcare providers [2,9]. The ability to collect pathogen and biomarker information on the spot enables rapid and accurate detection and stratification of sepsis, leading to the administration of appropriate therapeutics for sepsis (Figure 1). Performing these tests in a POC setting eliminates the bottlenecks that arise from transporting samples to and from laboratories, as well as the limited availability of highly trained laboratory personnel to perform these tests. Very importantly, thanks to their low cost, high sensitivity, rapid response, and potability, electrochemical-based POC devices are capable of high-frequency testing of clinically relevant biomarkers to study disease progression. Thus, POC diagnostic devices will greatly improve the speed at which a patient is diagnosed and receives treatment. This short review

**Table 1**

List of organisms causing sepsis. Any type of infection can lead to sepsis. This includes bacterial, viral, or fungal infections.

<table>
<thead>
<tr>
<th>Organisms causing sepsis</th>
<th>Gram-positive</th>
<th>Gram-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria [10–15]</strong></td>
<td>• Methicillin-resistant Staphylococcus aureus (MRSA, mortality rate of 15–60% [10,11]) • Methicillin-sensitive Staphylococcus aureus (MSSA) • Staphylococcus epidermidis, Staphylococcus spp • Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae (leading cause of neonatal and maternal sepsis [12,13,15]), Streptococcus dysgalactiae spp equisimilis (SDSE), Streptococcus anginosus, Streptococcus constellatus, Streptococcus spp • Enterococcus faecalis, Enterococcus faecium, Enterococcus spp • Clostridium difficile, Clostridium perfringens, Clostridium tetani, Clostridium spp</td>
<td>• Escherichia coli (most frequent in some studies, &gt;20% [10,14]) • Moraxella catarrhalis • Neisseria meningitidis, Neisseria spp • Acinetobacter baumannii, Acinetobacter spp • Aeromonas hydrophila, Aeromonas spp • Bacteroides fragilis, Bacteroides spp • Burkholderia cepacia, Burkholderia spp • Citrobacter freundii, Citrobacter spp • Enterobacter spp • Haemophilus influenzae • Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella spp • Legionella pneumophila, Legionella spp • Pseudomonas aeruginosa, Pseudomonas spp • Proteus mirabilis, Proteus spp • Rickettsia spp • Salmonella enteritidis, Salmonella spp • Serratia marcescens, Serratia spp • Stenotrophomonas maltophilia, Stenotrophomonas spp • Vibrio vulnificus, Vibrio cholerae, Vibrio spp</td>
</tr>
<tr>
<td><strong>Viruses [15–19]</strong></td>
<td>• Herpes simplex virus (most common viral causes of neonatal sepsis [15]) • Human parechovirus (most common causes of viral sepsis in young children [17]) • Enterovirus (most common causes of viral sepsis in neonates and young children [15,17]) • Influenza virus • Dengue virus (leading cause of sepsis in southeast Asia) • Adenovirus • SARS-CoV-2</td>
<td></td>
</tr>
<tr>
<td><strong>Fungi [5,10,20]</strong></td>
<td>• Aspergillus spp, • Candida albicans, Candida parapsilosis, Candida tropicalis, Candida krusei, Candida spp (Candida species are the predominant cause of fungal sepsis [20])</td>
<td></td>
</tr>
</tbody>
</table>
highlights the latest advances in electrical- and electrochemical-based POC devices used for the sensitive and rapid detection of plasma-circulating protein biomarkers, cell-surface biomarkers, and bacteria that are indicative of sepsis. While the title refers to electrochemical devices, this review includes both electrical/impedance-based methods and electrochemical methods where a redox reaction is carried out to produce the measured signal. Regarding biomarkers, we have focused on proteins due to the wide variety of options and our special interest in multiplexed devices. Likewise, we have included cell-surface biomarkers to highlight the challenge of detecting cells and proteins from the same sample. Of the pathogens, we have focused on bacteria, since they are much more prevalent in leading to sepsis, and their culture-free detection at low concentrations is of particular interest. A summary of all these findings can be seen in Table 2.
Electrochemical-based devices for protein biomarkers

A sepsis biomarker must complement already-available information, such as patient history, physical examinations, and routine investigations (e.g., white blood cell count). It must also be able to differentiate sepsis quickly and accurately from sterile causes of overlapping diagnostic criteria. Several biomarkers have been found to be associated with sepsis, including procalcitonin (PCT), C-reactive protein (CRP), interleukins (IL-6, IL-8, IL-10), white blood cells (neutrophil to lymphocyte ratio (NLR) and CD64 expression on neutrophils (nCD64)) [7,21]. No single biomarker is specific enough to predict sepsis, therefore this section focuses on devices capable of multiplexing several biomarkers.

In particular, we will focus this section on devices capable of detecting plasma-circulating protein biomarkers and/or cell-surface biomarkers to highlight the challenge of detecting cells and proteins from the same sample. Electrical technologies that detect protein biomarkers include impedance spectroscopy and Coulter counter-based technologies (Figure 1).

Ingber’s laboratory investigated the coating of electrodes with reduced Graphene-Oxide nanoflakes (rGOx) to address the difficulties of multiplexed analysis of proteins in whole blood samples [22–24]. They noted that the high susceptibility of electrochemical sensing elements to biological fouling is one of the reasons behind the limited commercial success of affinity-based electrochemical sensors in complex biological samples. To address this issue, they coated electrodes with a 3D nanocomposite containing cross-linked bovine serum albumin doped with conductive nanomaterials, such as gold nanowires [22]. Eventually, they replaced gold with rGOx to dramatically reduce the cost of their device [23]. This approach was used to develop a device with four individual working electrodes and demonstrate the simultaneous detection of four sepsis biomarkers (PCT, CRP, syndecan-1, and PAMPs) in whole blood [23]. The system was found to avoid fouling during exposure to blood for 60 min. With promising results from an initial prototype microfluidic sensor, the project was further developed into the startup StataDX. As of 2022, the team is integrating simultaneous detection and quantification capabilities of up to 30 biomarkers [24].

Muthukumar and Prasad developed the DETecT sepsis (Direct Electrochemical Technique Targeting Sepsis), a portable device based on electrochemical impedance spectroscopy and on a metal/semi-conductor sensor interface (zinc-oxide coated electrodes to attain high sensitivity) for the simultaneous detection of sepsis biomarkers [28,29]. In this approach, the target analyte binds to the specific capture probe antibody within the double layer, leveraging antibody-antigen affinity mechanism across each working electrode. While version 1.0 of this device demonstrated the simultaneous detection of five cytokines (IL-6, IL-8, IL-10, TRAIL & IP-10) in plasma [28], version 2.0 added 3 additional biomarkers (d-dimer, CRP and G-CSF), demonstrating the 16 working electrodes system with whole blood samples [29]. The DeTecT sepsis 2.0 device, integrated the simultaneous detection of eight cytokine biomarkers with a machine algorithm to predict the outcomes. To avoid interference from whole blood components, the optimized device used shorter analysis times, higher sample volume (~ 100 µL) and a smaller sample frequency range.

Coulter-counter-based assays demonstrate unique potential for multiplexed electrical evaluation of plasma-circulating proteins and cell-bound proteins. Our laboratory has reported a differential count-capture technology used in a microfluidic platform to enumerate leukocytes and quantify nCD64 levels from a small whole blood sample [25]. In this approach, a microfluidic capture chamber with entrance and exit electrodes enumerated the total captured cells with differential counting. The specificity of this method is based on the use of functionalized antibodies in the capture chamber. In subsequent work, we demonstrated that the same platform could also be used to detect plasma-circulating proteins, despite size differences between cells and proteins, by using sandwich immunoassays on the surface of micro-sized beads [30–32]. Our team first demonstrated detection of one protein [30] and was soon able to multiplex the system by incorporating the use of commercially available [31] and in-house synthesized [32] micro-sized beads with unique, distinguishable electronic signatures. In collaboration with the Han laboratory, our team introduced novel precision-engineered microparticles that achieved enhanced electrical multiplexing. Droplet microfluidic synthesis yielded highly monodisperse populations of magnetic hydrogel beads with the properties required for functionalization and multiplexed electrical counting. Each population of beads was designed to contain a different amount of hydrogel material, resulting in a unique electrical impedance signature. By incorporating hydrogel beads, the team demonstrated the detection of protein biomolecules and DNA targets.

Electrochemical-based devices for detection of bacteria

Quickly and accurately identifying pathogens that cause sepsis in clinical samples and at the point-of-care has been a challenging goal. High sensitivity, short time-to-results, and low cost are crucial components for POC devices. Specifically, we will focus this section on devices capable of detecting bacteria, since bacterial infections cause most cases of sepsis, and their culture-free detection at low concentrations is of special interest. Electrical technologies that detect bacteria include electrochemical and culture-free approaches (Figure 1).
<table>
<thead>
<tr>
<th>Technology</th>
<th>Target(s)</th>
<th>Sample medium</th>
<th>Metrics</th>
<th>Clinical samples</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis Biomarkers</td>
<td>IL-6</td>
<td>Unprocessed plasma</td>
<td>LOD: 23 pg/mL</td>
<td>NO</td>
<td>[22]</td>
</tr>
<tr>
<td>3D nanocomposite doped with</td>
<td></td>
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<tr>
<td>conductive nanomaterials for</td>
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<tr>
<td>Electrochemical Biosensor</td>
<td></td>
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<tr>
<td>Graphene Enabled Affinity-based</td>
<td>PCT</td>
<td>Undiluted serum: PCT</td>
<td>PCT (LOD and WR) serum: 64.5 pg/mL, 0.09 – 10.24 ng/mL</td>
<td>21 serum samples (PCT test only)</td>
<td>[23]</td>
</tr>
<tr>
<td>Electrochemical Biosensor</td>
<td>CRP</td>
<td>50% whole blood: All</td>
<td>whole blood: 24.7 pg/mL, 0.07 – 2.49 ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PAMPs syndecan-1</td>
<td>targets</td>
<td>CRP (LOD and WR): 0.492 μg/mL, 0.63 – 3.76 μg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrochemical Impedance</td>
<td>IL-6, IL-8, IL-10, TRAIL, IP-10</td>
<td>Undiluted Plasma</td>
<td>IL-6 (LOD and WR): 0.1 pg/mL, 0.01 – 10^5 pg/mL</td>
<td>20 septic and 20 nonseptic plasma samples</td>
<td>[28]</td>
</tr>
<tr>
<td>Spectroscopy Device</td>
<td></td>
<td></td>
<td>IL-8 (LOD and WR): 0.1 pg/mL, 0.1 – 5x10^3 pg/mL</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>TRAIL (LOD and WR): 1 pg/mL, 1 – 10^3 pg/mL</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>IP-10 working range: 1 – 2x10^3 pg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocapture Biochip Platform</td>
<td>CD64</td>
<td>Whole Blood</td>
<td>None given</td>
<td>30 septic and 10 healthy whole blood samples</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sensitivity 100%; Specificity 75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>IL-6</td>
<td>Plasma</td>
<td>LOD: 122 pg/mL, working range: 10^2 – 10^5 pg/mL</td>
<td>15 plasma samples from potentially septic patients</td>
<td>[30]</td>
</tr>
<tr>
<td>Electroactive RNA-cleaving DNAzyme assay</td>
<td>E. coli</td>
<td>Buffer and Urine</td>
<td>LOD and WR (both): 10^2 CFU/mL, 10^5 – 10^10 CFU/mL</td>
<td>NO</td>
<td>[31]</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[33]</td>
</tr>
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</table>

(continued on next page)
Other approaches include electrical susceptibility testing [26,27].

Soleymani’s team developed a set of culture-free approaches to electrically quantify bacteria from clinical urine samples in <1 h [33,34]. This approach integrated RNA-cleaving DNAzymes specific to protein targets released by *E. coli* into an electrical chip with nanostructured electrodes. Interactions between the DNAzyme and the bacterial proteins resulted in cleavage of the DNAzyme to release a detectable DNA barcode. The initially achieved LOD (10^3 CFU/mL, *E. coli* in 10 μL of urine) [33], was improved to 138 CFU/mL in unprocessed urine by developing a microgel magnetic bead assay with the DNAzymes [34].

Merkoci’i lab explored different electrochemical approaches for identifying bacteria [35,36]. They first used AuAg nanoshells as electrochemical reporters to detect *E. coli* and *S. typhimurium* within 10 min [35]. They were able to electrically detect the bacteria without the use of biological receptors, and instead used the catalytic properties of the nanoshells to nonspecifically interact with the highly differentiated bacterial cell surfaces. Once bound to the cells, the aggregates of nanoshells were detected using screen-printed carbon electrodes. This team also used the electrochromic properties of polyaniline for the visual detection of *E. coli* [36]. The color of the assay was based on the different oxidation states of electropolymerizing polyaniline on an ITO screen-printed electrode. A constant potential was applied to the working electrode and the presence of *E. coli* captured by antibodies on the electrode surface increased the electrical resistance, preventing the triggering of the electrochromic behavior. Although the device was applied to water monitoring, the authors believe that it could also be adapted for use with clinical samples.

Goel’s lab developed a lab-on-chip platform for the simultaneous culture and electrochemical detection of bacteria [37]. They integrated a microfluidic chamber with screen-printed electrodes and laser-induced graphene heaters to create the temperatures required for bacterial culture. Cyclic voltammetry and chronoamperometry techniques, were employed to investigate the growth of bacteria. The device achieved an LOD of 2 × 10^3 CFU/mL and decreased the time for incubation by replacing typical bacterial incubation with microfluidics.

Functionalized electrodes with DNA capture probes have also been used to create electrochemical sensors capable of the analysis of *S. aureus, E. coli, Pseudomonas aeruginosa*, and *Proteus mirabilis* spiked in whole blood samples within 1 h [38,39]. The device consists of 16 gold sensors, where the central working electrode was functionalized with thiolated DNA capture probes. The
approach used a sandwich binding scheme with a capture probe and a DNA detector probe (bound with HRP) for detecting the bacterial 16S ribosomal RNA (rRNA). Afterward, a detector probe with horseradish peroxidase is incubated. The device achieved a sensitivity of $10^4$ CFU/mL, which limits its use in cases of sepsis which can be caused by lower concentrations.

**Current and future challenges**
While electrochemical point-of-care devices exist for the separate identification of plasma-circulating biomarkers (including IL-6, CRP and PCT) [23,28–30], cell-surface biomarkers (including CD64) [25], and bacteria from clinical samples [33,34] there are still several areas where point-of-care diagnostics for sepsis need to improve before being usable.

- **Multiplexed detection**: Although cell counts, cell-surface biomarkers, and plasma-circulating proteins can monitor and track host immune response, acquiring these metrics using one device and a single sample, throughout the patient’s entire hospital stay is challenging. To the best of our knowledge, as of now, there are no available devices that can detect cell-surface and plasma-circulating biomarkers from a single blood sample. This lack of technology limits the potential avenues for multiplexing and diagnosis, as obtaining a complete and comprehensive understanding of a patient’s condition requires multiple tests. For example, it has been shown that a combined measure of CRP and CD64+ cells greatly improves sepsis diagnosis [40], however, currently, this would need to be done using two separate tests. Obtaining a patient’s detailed biomarker “fingerprint” would allow for clear, actionable diagnosis and successful individualized treatment. Coulter counter-based technologies that can work with whole blood samples have the potential to overcome these limitations.

- **Need for an integrated system**: The milieu of biomarkers is known to change as the infection progresses and the patient recovers or worsens. Likewise, there are too many biomarkers for the clinician to properly use them as individual numbers to predict a patient’s response to treatment. So, we need an integrated system, probably powered by AI, to give a predictive score or number to the doctor to guide bedside treatment.

- **Sensitive detection from whole blood**: Few point-of-care technologies have demonstrated their ability to provide sensitive detection of biomarkers directly from whole blood as opposed to plasma. Since processing blood into plasma requires a separation device, this step reduces device applicability to point-of-care testing. While work has been done with multiplexed detection of protein biomarkers in 50% whole blood at elevated PCT concentrations [23], more sensitive platforms that can detect biomarkers in whole blood in clinically relevant ranges are needed. For example, studies have reported that circulating levels of three of the most well-known biomarkers, IL-6, CRP and PCT, can range from 1 to 14 pg/mL [41], <8.7 mg/dL [42], and <0.78 ng/mL [43] in healthy patients to <0.1–305 ng/mL [44], >8.7 mg/dL [42], and 4–53.5 ng/mL [43] in septic patients, respectively. Other possible solutions include the incorporation of a microfluidic module in point-of-care devices for the separation [45,46] and/or extraction of plasma [47].

- **Culture-free detection of bacteria**: Because bacterial concentrations <10 CFU/mL are often sufficient to cause infection, especially in infants [48,49], it is difficult to achieve properly sensitive detection without culturing blood first. Multiplexed testing that utilizes positive blood cultures (i.e., after the blood culture steps) currently exists with the FDA-approved Biofire FilmArray Blood Culture Identification panel, which is capable of simultaneously testing for over 40 pathogen targets using PCR amplification and optical detection techniques [50,51]. Likewise, while culture-free bacterial detection in urine samples (electrochemical readout) [33,34], and in whole blood samples (optical readout) [52] have been reported, they detect only one pathogen at a time. The next step is to achieve culture-free electrochemical and multiplexed detection of bacteria at the point-of-care, as multiplexed detection is essential due to the complexity of sepsis [48]. The FDA-approved T2 Bacteria Panel (T2 Biosystems) has demonstrated 3–5 h multiplexed detection of the 5 most common sepsis-causing bacteria (E. coli, S. aureus, K. pneumoniae, P. aeruginosa, E. faecium) which account for 61.4% of sepsis cases at a 2–11 CFU/mL sensitivity from a whole blood sample using nucleic acid amplification and magnetic resonance [53]. However, detection is not possible at the point-of-care, and while the T2Bacteria panel quickly and accurately diagnoses bloodstream infections (BSIs) caused by 5 bacteria in the panel, one study found that 40% of the samples tested (58/146 patients with negative blood culture and positive T2Bacteria results) did not meet criteria of probability of possible BSI, and samples were defined as presumed false positives [54]. Therefore, the next step on this front would be miniaturization and the addition of multiplexing capabilities to the reported culture-free bacteria detection methods.

- **Large volume processing**: Due to the low bacterial concentrations that can cause sepsis, reliable detection without culturing requires a large quantity of blood, necessitating point-of-care systems capable of processing mL quantities in a few hours or less. Currently, the T2 Bacteria Panel processes 3–4 mL of blood [55] demonstrating the value of large blood volumes in sensitive culture-free assays for bacteria. Because blood volume is not a significant limitation in adults (as 4–5 mL are drawn at once), sensitive
detection should be prioritized over using low volume tests.

Conclusions
This short review paper reports on the current state of electrical and electrochemical point-of-care devices for the diagnosis of sepsis. This manuscript describes novel and attractive electrical and electrochemical techniques, discusses the challenges in identifying the different sepsis biomarkers, and proposes areas of research to overcome these limitations.

Declaration of Competing Interest
The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Rashid Bashir declare financial interests in Prenosis, Inc. and LabSimply, Inc.

Data availability
No data was used for the research described in the article.

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References
Papers of particular interest, published within the period of review, have been highlighted as:* of special interest ** of outstanding interest


This paper provides updated definitions and clinical criteria for sepsis.


In this paper the authors noted that the high susceptibility of electrochemical sensing elements to biological fouling limits the commercial success of affinity-based electrochemical sensors in complex biological samples. They address this issue by coating electrodes with a 3D nanocomposite containing cross-linked bovine serum albumin doped with conductive nanomaterials.


This platform enables detection of multiple sepsis biomarkers simultaneously and dramatically reduce the cost of the device reported in [26] by replacing gold with rGOx.

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Demonstration of the differential count-capture technology to enumerate leukocytes and quantify nCD64 levels from clinical whole blood samples.


Demonstration of the DETect sepsis device for the simultaneous detection of five cytokines in plasma samples.


Demonstration of a culture-free approach to electrically quantify bacteria from clinical urine samples.


