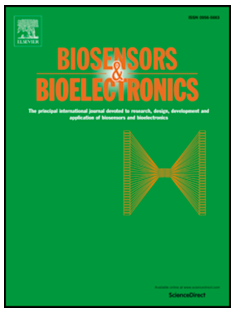


Point-of-care diagnostics for sepsis using clinical biomarkers and microfluidic technology

Zoe Bradley, Nikhil Bhalla

PII: S0956-5663(23)00123-9

DOI: https://doi.org/10.1016/j.bios.2023.115181

Reference: BIOS 115181

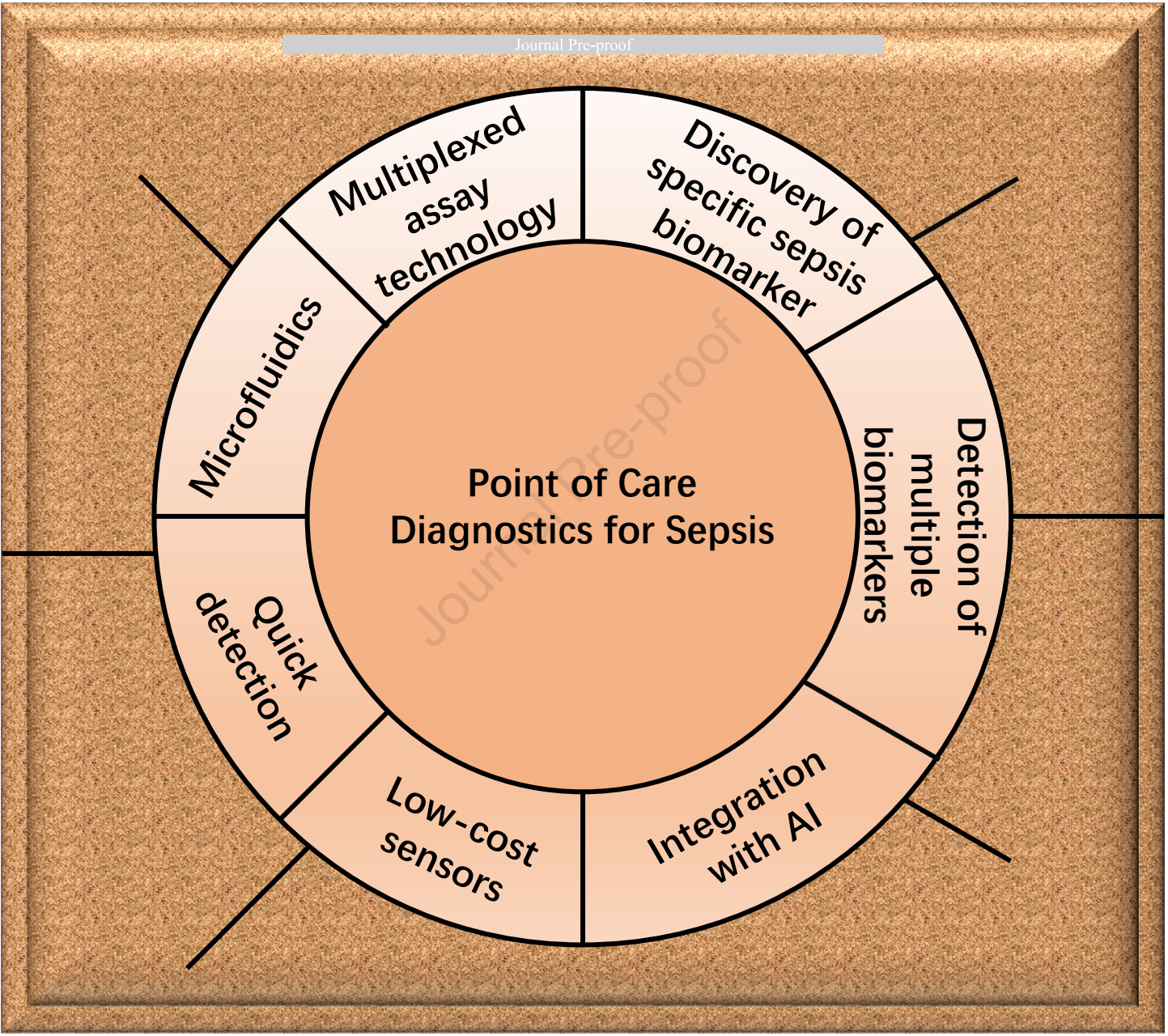
To appear in: *Biosensors and Bioelectronics*

Received Date: 18 November 2022

Revised Date: 13 February 2023

Accepted Date: 23 February 2023

Please cite this article as: Bradley, Z., Bhalla, N., Point-of-care diagnostics for sepsis using clinical biomarkers and microfluidic technology, *Biosensors and Bioelectronics* (2023), doi: https://doi.org/10.1016/j.bios.2023.115181.



Point-of-Care Diagnostics for Sepsis using Clinical Biomarkers and Microfluidic Technology

Zoe Bradley[a], Nikhil Bhalla[a,b]

[a]*Nanotechnology and Integrated Bioengineering Centre (NIBEC), School of Engineering, Ulster University, Ulster University, 2-24 York Street, Belfast, BT15 1AP, United Kingdom*
[b]*Healthcare Technology Hub, School of Engineering, Ulster University, Ulster University, 2-24 York Street, Belfast, BT15 1AP, United Kingdom*

Abstract

Sepsis is a life-threatening immune response which is caused by a wide variety of sources and is a leading cause of mortality globally. Rapid diagnosis and appropriate antibiotic treatment are critical for successful patient outcomes; however, current molecular diagnostic techniques are time-consuming, costly and require trained personnel. Additionally, there is a lack of rapid point-of-care (POC) devices available for sepsis detection despite the urgent requirements in emergency departments and low-resource areas. Recent advances have been made toward developing a POC test for early sepsis detection that will be more rapid and accurate compared to conventional techniques. Within this context, this review discusses the use of current and novel biomarkers for early sepsis diagnosis using microfluidics devices for POC testing.

Keywords: Sepsis biomarkers, Sepsis diagnostics, Microfluidic technology, Microfluidic materials, Point-of-care testing

1. Introduction

Sepsis is defined as the dysregulation of a defensive immune response that is triggered by an infection from bacteria, viruses, fungi, or parasites Sweeney et al. (2019); Ghazal et al. (2022). Sepsis is a leading cause of mortality globally Vignon et al. (2020), accounting for 4-17% of Intensive Care Unit (ICU) admissions with a 17-26% death rate in high-income countries. Middle to low-income countries have reported mortality rates as high as 80% concerning sepsis Yuk et al. (2018). A major cause for the high mortality rates associated with sepsis is due to challenges in relation to detecting early symptoms and initiating correct treatment Claxton et al. (2020). Every hour of sepsis treatment delay accounts for an 8% increased risk of mortality in patients suffering from septic shock McGregor (2014). Initial diagnoses often take place in primary care, however, limited types of diagnostic tests are

Email addresses: z.woods@ulster.ac.uk (Zoe Bradley), n.bhalla@ulster.ac.uk (Nikhil Bhalla)

used, and many cases are missed and are then detected at a later stage in the hospital, with associated poor healthcare outcomes Loots et al. (2017); Morris et al. (2017).

Sepsis has a different host response compared to typical localized microbial infections and due to its dynamic and acute nature, it requires immediate medical treatment Min et al. (2018). Microbiological cultures are the gold standard for sepsis detection and guiding antibiotic treatment. Microbiological cultures aim to detect bacteria, viruses, or fungi in the blood Cohen et al. (2015), and this can be done through approaches including nucleic-based systems such as polymerase chain reaction (PCR) den Brand et al. (2018) and culture-based systems such as blood cultures, which are the most used amongst clinicians. However, microbiological cultures lack sensitivity and have long incubation times of up to 72 hours Papafilippou et al. (2020) and by the time a positive result is confirmed, the patient may already have developed septic shock or organ failure. Another major challenge for healthcare professionals is the diagnosis of sepsis in critically ill patients due to inflammation in the body from other infections and prior use of antibiotics which can lead to false negative results Vincent (2016). Certain bacteria have low microbial activity signals which can also lead to false negative results and unfortunately, 30-40% of predicted sepsis cases are found to be culture-negative Cohen et al. (2015). Additionally, it may be difficult to achieve sufficient blood volume for culture samples from patients who have low blood pressure Fang et al. (2021). Current sepsis screening methods rely on symptom assessment tools such as the sequential organ failure assessment (SOFA), the systemic inflammatory response syndrome (SIRS) guidelines, and the quick SOFA (qSOFA) score Fang et al. (2021). Symptoms include lower blood pressure, increased white blood cell levels, high respiratory rate, and confusion Durkin et al. (2021). However, screening tools often fail to meet the accuracy and speed required in sepsis management and cannot determine the cause or stage of infection. The challenges surrounding the gold-standard blood culture for sepsis detection and the lack of predictive value of screening tools have proven that a superior method is urgently required.

2. Sepsis Biomarkers

Biomarkers are classified into one of the following categories as shown in Fig. 1: acute phase proteins like C-Reactive Protein, Procalcitonin, and Serum Amyloid A, proinflammatory cytokines such as interleukin-6, biomarkers of activated neutrophils and monocytes, for example, CD64, infectious organisms and related protein, receptors, anti-inflammatory markers, and biomarkers for organ dysfunction Hung et al. (2020). There are more than 100 biomarkers reported for use in sepsis indication, and they can differentiate between bacteria, viruses, and fungal infections, which can also indicate the difference between a local infection and sepsis Teggert et al. (2020). Biomarkers already play important roles in guiding antibiotic therapy, predicting sepsis complications, and evaluating the difference between gram-positive and gram-negative microorganisms Pierrakos and Vincent (2010). Recent studies have proposed the use of biomarkers as an alternative to blood cultures for rapid sepsis diagnosis to allow for improved patient outcomes due to earlier and more specific therapeutic interventions in sepsis treatment Alba-Patino et al. (2020). Table 1 summarises common and emerging clinical biomarkers for sepsis detection.

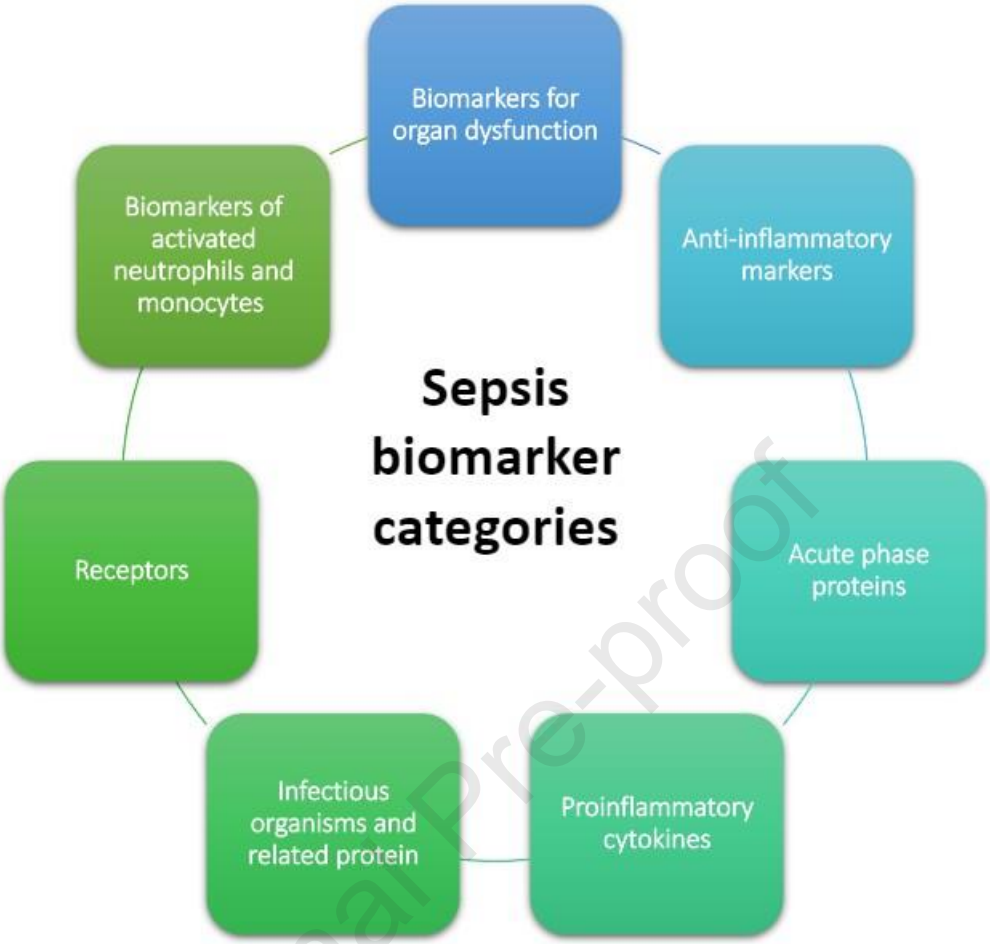


Figure 1: Schematic showing various sepsis biomarker categories.

2.1 C-Reactive Protein

C-reactive protein (CRP) is a commonly researched acute-phase protein. It belongs to the pentraxin family of ligand-binding plasma proteins used for sepsis diagnosis, which is triggered by both inflammation and infection Pant et al. (2021). CRP is initially expressed in the liver and production is induced by interleukin (IL)-6, IL-1β, and TNF-α. Normal CRP levels are <10 μg/mL but levels can peak at 40-200 μg/mL once secretion commences 4-6 hours after stimulation. Peak levels are reached at 36-48 hours after stimulation. The disadvantage of CRP is its low specificity as levels rise in the instance of other non-inflammatory diseases such as trauma, pancreatitis, or burns and has been restricted in sepsis diagnosis due to this Pant et al. (2021); Eschborn and Weitkamp (2019). Another major drawback with CRP detection is the prevalence of the hook effect which is caused by excess target antigens that disturb the sandwich immunoassay on the test line and cause a false negative result despite a high concentration of target antigen Ross et al. (2020). Fig. 2 shows a graphical representation of CRP hook effect, as antigen concentrations increase, instead of the test line colour intensity continuing to increase, it starts to decrease. Previous studies have focused on overcoming the hook effect. Rey et al., investigated how the use of an extra filter pad can control the time release of the detection antibody in a paper-based lateral flow device.

The pad is strategically placed between the conjugate and nitrocellulose membrane and pretreated with sucrose. The automatic time release helped to reduce the hook effect in the CRP assay Rey et al. (2017). Another study by Oh et al., produced a paper-based immunochromatographic assay (HEF-ICA) which also delayed the detection antibody release from the conjugate pad by applying a bridged sample pad to the design which was able to overcome CRP hook effect Oh et al. (2018). These previous studies were able to overcome the hook effect associated with CRP, but they only achieved a single biomarker assay and CRP has not been recommended as a standalone test for sepsis detection as levels are suppressed in the presence of steroids, it has a slow response to stimulus, and is not specific Mysler et al. (2004).

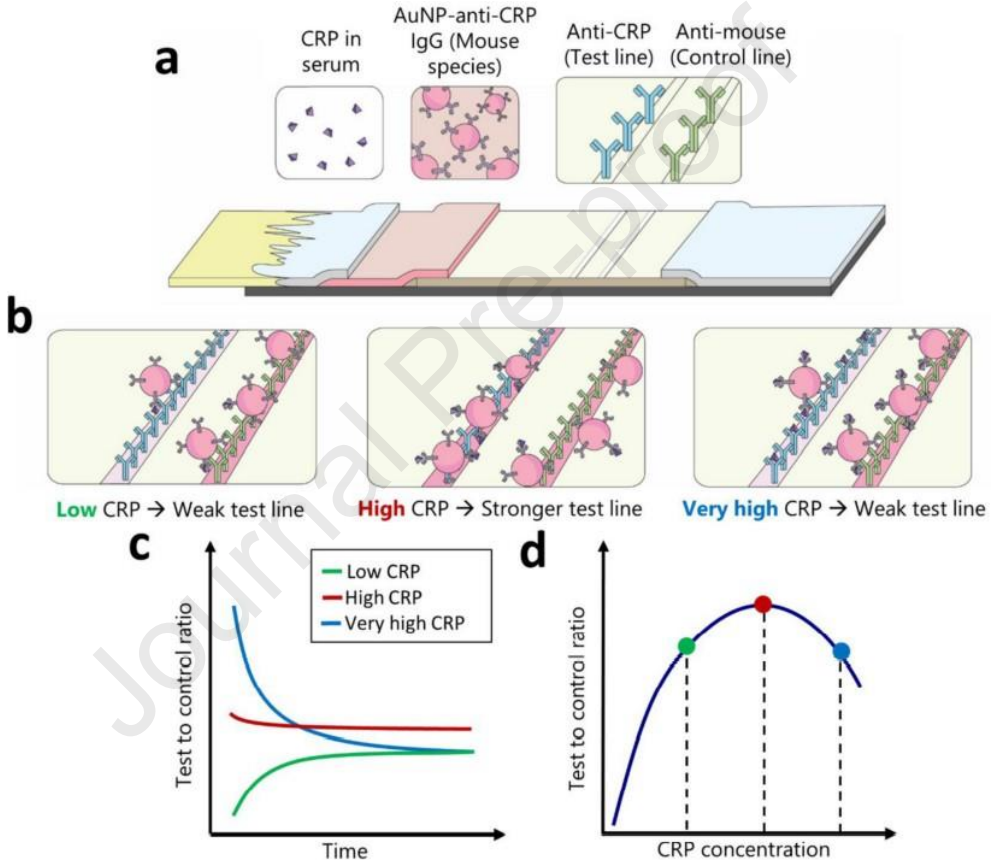


Figure 2: Lateral flow assay with CRP hook effect schematic (a) Lateral flow strip schematic (b) Low to high CRP signal development (c) CRP kinetic development of test to control ratio for low, high and very high CRP concentrations (d) final test to control ratio for low, high, and very high CRP concentrations. Reproduced from ref. Rey et al. (2017) with permission from ACS Publications, copyright 2017.

2.2 Procalcitonin

Procalcitonin (PCT) is a prohormone of calcitonin and is also an extensively reported biomarker associated with sepsis Shiferaw et al. (2016). PCT is released during systemic inflammation caused by a bacterial infection, with the liver producing the largest amount of PCT during sepsis, plasma concentrations can reach up to 1000-fold. PCT synthesis is stimulated by similar cytokines such as IL-6, and IL-1β Eschborn and Weitkamp (2019). PCT secretion begins 2-4 hours after the onset of infection, peaks at 12-24 hours, and has a half-life of 24 hours Mussap et al. (2007).

PCT levels remain high as the inflammatory process continues and levels correlate directly with the sepsis severity, allowing PCT to discriminate between patients with infectious and non-infectious systemic inflammation. PCT has greater specificity compared to CRP and studies have shown that increased PCT levels upon admission to an ICU are related to an increased risk of severe sepsis or septic shock. PCT values can help to administer antibiotics and help to reduce over administration of antibiotics Raveendran et al. (2019).

2.3 Interleukin-6

IL-6 is a cytokine protein from the glycoprotein-130 (gp130) family which is multifunctional as it can act as a pro or anti-inflammatory cytokine Zarogoulidis et al. (2013). IL-6 is an emerging biomarker for sepsis detection and normal levels of IL-6 are <25 pg/mL with elevated levels reaching >1000 pg/mL which is associated with septic shock and mortality Damas et al. (1992). IL-6 has recently been proposed as an important biomarker of interest for sepsis diagnosis as unlike CRP and PCT, IL-6 increases rapidly after a few minutes of stimulation and has a half-life of 1 hour Weidhase et al. (2019). Due to the fast dynamics associated with IL-6, recent studies have focused on detecting IL-6 rather than CRP and PCT. Alba-Patino et al., proved that monitoring small variations in IL-6 levels is critical for rapid sepsis detection and could detect IL-6 from a whole blood sample using a paper-based biosensor with a limit of detection (LOD) of 0.1 pg/mL in 17 minutes Alba- Patino et al. (2020). However, previous studies have found it difficult to obtain a low enough LOD that would be beneficial in a healthcare setting.

2.4 Serum Amyloid A

Serum Amyloid A (SAA) is an acute phase inflammatory biomarker in charge of the transportation of cholesterol to the liver to ensure correct bile secretion, inducing enzymes and recruiting immune cells into inflammatory sites Cetinkaya et al. (2009). SAA is produced by hepatocytes, but new studies have discovered that it is also synthesized by adipocytes and its concentration in serum is related to body mass index (BMI) Yang et al. (2006). SAA has been more widely researched for equine and feline sepsis Barr and Nieman (2022); Troèt al. (2017) but it is gaining interest in human diagnostics as it can show as much as a 1000-fold increase in sepsis infection after 8-24hrs of onset from inflammation. SAA has shown favourable kinetic results in response to acting as a neonatal biomarker, but further research is required to evaluate how gestational age and birth weight affect the infant's ability to secrete SAA in response to sepsis Bengnér et al. (2021). SAA is an emerging biomarker for sepsis detection, it has been tested within multiplex assays of recent research, but not yet as a standalone biomarker for human sepsis detection.

2.5 Neutrophil CD64

Neutrophils are important in response to sepsis with previous studies showing changes to neutrophil mechanics, motility, and morphology during sepsis. Neutrophil CD64 is a Fc*y* receptor distributed on the surface of monocytes, macrophages and dendritic cells and is not found on neutrophils under normal physiological conditions Cong et al. (2021). CD64 levels increase rapidly to 5-10 times the normal level on neutrophils in the instance of sepsis or bacterial infection within 1-6 hours Eichberger

et al. (2022).

CD64 is a promising biomarker for bacterial sepsis with a recent meta-analysis using results from 2471 patients to compare the accuracy of PCT, CRP, and CD64 for use as a sepsis biomarker found that CD64 outperformed both CRP and PCT in the detection of bacterial sepsis Yeh et al. (2019). On the other hand, Tang et al. found that CD64 only performed better during the early stages of sepsis diagnosis and PCT had more diagnostic value in the later stages of sepsis, concluding that a multiplex tool was required for optimum diagnostic accuracy Tang et al. (2018).

3. Microfluidics for Point-of-Care testing

3.1 Multiplexed Sepsis Assays

Despite recent efforts, there is no gold standard sepsis biomarker that can be used as a single tool for accurate diagnosis, proposed by Ng in 2004. An ideal biomarker for sepsis should have characteristics such as being able to rise quickly in response to stimulation, have a prolonged elevation to ensure detection and reduce the requirement for repeat testing. These characteristics reduce the requirement for excessive use of antibiotics and ensure optimal patient outcomes Ng (2004). Complexities and variable times of individual biomarkers make using a single biomarker for sepsis diagnosis inappropriate and unreliable, especially in those with an underlying health condition or in a critically ill state as other health conditions and antibiotic use can lead to a misinterpretation of biomarker results, reducing specificity. A trend in recent studies agreed that a combination of biomarkers is needed for accurate sepsis diagnosis. Bengner et al. suggested pairing a fast-acting biomarker like IL-6 with a highly sensitive biomarker like PCT or CRP for a more rapid and sensitive POC test for sepsis detection Bengnér et al. (2021). However, more research is required to determine an optimal biomarker combination as it is unlikely that there will ever be a single biomarker for sepsis diagnosis due to its heterogeneous nature therefore, a multiplexed approach should be considered Patel and McElvania (2019). Another challenge for healthcare professionals is the lack of validated clinical results which limits potential biomarker assays from being used in the real world.

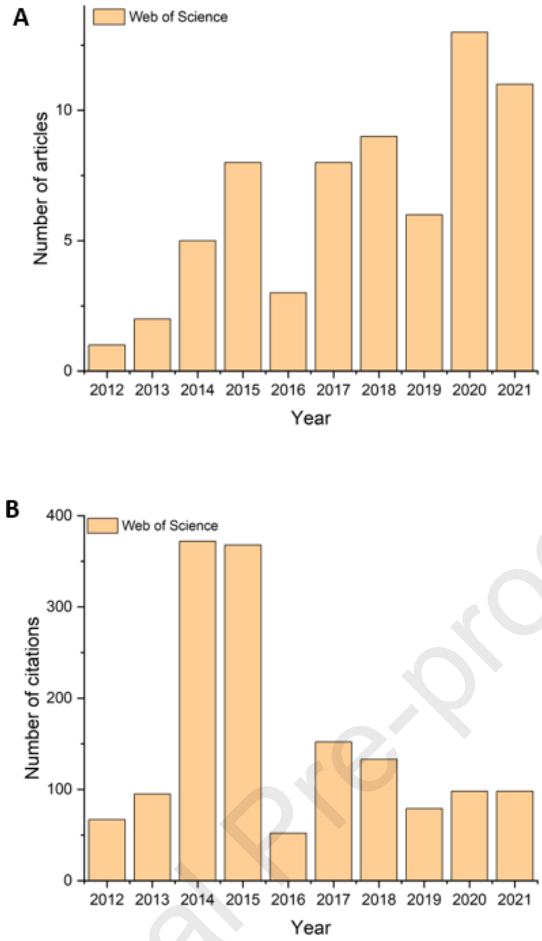


Figure 3: Documents on microfluidics and sepsis in web of science and scopus in last 10 years A) number of articles and B) number of citations.

A Point-of-Care (POC) test is an analytical test that provides the end user with a rapid medical diagnosis in real time and is particularly important in emergency departments or in areas with limited resources, such as developing countries Drain et al. (2014). POC testing is required in various fields to prevent infectious diseases or foodborne illnesses through biomarker monitoring. The World Health Organization (WHO) released "ASSURED" guidelines which state that a faultless POC test should be affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable Naseri et al. (2022). Previous studies have focused on reducing the sample preparation phase for POC testing to enable the end user to test a sample without any pretreatment steps. This is a major advantage compared to conventional blood testing and simple methods to eliminate sample pretreatment steps have been explored, such as the study by Guo, Hansson, and Van der Wijngaart who used synthetic paper to filter plasma from a whole blood sample which can be built into a paper-based microfluidic device and act at the POC Guo et al. (2020). Microfluidic technology allows the testing of multiple biomarkers simultaneously, known as 'multiplexing' within automotive laboratory equipment, in vitro devices, and drug screening technology. The coupling of microfluidics and biosensors has gained recent interest as attractive POC testing devices as they are portable due to their small size, require low blood sample volumes, are extremely accurate, and most importantly have short detection times Akyazi et al. (2018). Microfluidics also reduces human error and the possibility of false

positives through automated systems. Fig. 3 shows the number of sepsis and microfluidic articles and citations from Web of Science over the past 10 years. This shows that there is a growing interest in this field of work due to the demand of an alternative test for early sepsis diagnosis. It also shows that the use of microfluidic technology for sepsis diagnosis is gaining popularity.

Table 1: Clinical Biomarkers for Sepsis Detection

Sepsis biomarker	Category	Source	Normal concentration for healthy individuals	Severe sepsis concentrations	Peak onset time after stimulus	Sample type	Reference
CRP	Acute-phase protein	Liver	<3 µg/mL	>50 µg/mL	4-6 hours	Whole blood, serum and urine	Povoa et al. (2005) Shim et al. (2019) Ozdemir et al. (2020)
PCT	Acute-phase protein	Thyroid gland	<0.05 ng/mL	>2 ng/mL	12-24 hours	Whole blood and serum	Shiferaw et al. (2016) Shim et al. (2019)
SAA	Acute-phase protein	Liver	<10 µg/mL	>1 mg/mL	8-24 hours	Whole blood and serum	Cetinkaya et al. (2009)
IL-6	Pro or anti-inflammatory cytokine	Monocytes, endothelial cells, and adipose tissue	<25 pg/mL	>1000 pg/mL	6 hours	Whole blood, serum and cerebrospinal fluid	Eichberger and Resch (2022) Lenski et al. (2017)
IL-8	Pro or anti-inflammatory cytokine	Macrophages	<10 pg/mL	>234 pg/mL	1-3 hours	Whole blood, serum and cerebrospinal fluid	Tanak et al. (2021) Hi-rao et al. (2000) Zhou et al. (2015) Liu et al. (2018)
CD64	Cell marker	Monocytes	<8 mcL	>800 mcL	24 hours	Whole blood and serum	Dimoula et al. (2013) Du et al. (2014)
Lactate	Other	Myocyte tissue	<2nmol/L	>3.9 mmol/L	24 hours	Whole blood, serum and urine	Nguyen et al. (2010) da Gomes Cunha et al. (2020) Junior et al. (2021)

3.2 Microfluidic Materials

A microfluidic device normally consists of an inlet, channel, mixer, valve, and outlet Mou and Jiang (2017). The material from which a microfluidic device is manufactured from is vital to performance. Table 2 summarizes microfluidic materials and their properties with table 3 outlining different immobilisation techniques for bioreceptors onto various microfluidic materials.

Table 2: Microfluidic Material Properties

Property	Silicon	Glass	Paper	Elastomer	Thermoset Plastic	Hydrogel
Optical transparency	None	High	Low	High	High	Low to medium
Hydrophobicity	Hydrophilic	Hydrophobic	Amphilic	Hydrophobic	Hydrophobic	Hydrophilic
Smallest channel dimension	< 100 nm	< 100 nm	< 200 µm	< 1 µm	< 100 nm	< 10 µm
Thermostability	Very high	Very high	Medium	Medium	High	Low
Solvent compatibility	Very high	Very high	Medium	Low	High	Medium

Traditionally glass and silicon were the materials of choice for a microfluidic device due to their availability however, these materials are expensive and brittle Fallahi et al. (2019). Paper is a common POC test material as it is low-cost, abundant, hydrophilic, and biocompatible. Self-driven capillary action is made possible by the porous cellulose matrix of paper, eliminating the need for extra fluid flow equipment such as pumps. Paper-based devices, such as lateral flow devices (LFDs) are simple to use and environmentally friendly compared to other materials Li and Steckl (2018). Paper-based microfluidic devices (μPADs) have gained interest in recent years and have encountered many innovative elements such as research conducted by Sun et al., who developed a novel origami style μPAD for the detection of CRP and prealbumin (PAB) from a sample of whole human blood. This device can separate blood cells from target analytes in 75 seconds and detect target analytes in the pg/mL range with a total assay time of 65 minutes Sun et al. (2022). Another innovative design was developed by Verma et al., who made a 3D μPAD to perform an enzyme-linked immunosorbent assay (ELISA). The technology targets CRP by using a sliding strip for the sensing area and an area around this which store and releases all assay liquid required. The result can be read visually for a qualitative measurement or quantitatively using a reader. This device is highly sensitive and can detect CRP in the dynamic range of 1-100 ng/mL from a blood sample. However, a downside to this is the accuracy is only 89% and has a long assay time of 90 minutes Verma et al. (2018).

Polymers polydimethylsiloxane (PDMS) and polymethylmethacrylate (PMMA) are also popular biocompatible materials used to make microfluidic devices. PDMS is an elastomer made from silicon that is flexible, has high gas permeability, and has excellent optical transmissivity Ariati et al. (2021). However, it is difficult to form microfluidic channels within PDMS material and challenges have arisen with sample absorption which has limited its use in commercial products. On the other hand, PMMA is an acrylic-based thermoplastic that has good stability, is chemically resistant, and is highly transparent, like glass but is far cheaper, lighter, and tough Vo and Chen (2022). Hassanpour-Tamrin, Sanati-Nezhad, and Sen combined PDMS and PMMA materials to form a low-cost hybrid PDMS-PMMA bond within a microfluidic device for use as a promising low-cost diagnostic device Hassanpour- Tamrin et al. (2021).

Another study produced a wave-shaped microfluidic chip (WMC) assisted multiplexed detection platform (WMC-MDP) made from PDMS to detect CRP, PCT, and IL-6 with LODs of 0.16 μL/mL, 0.1 ng/mL, and 12.5 mg/mL, respectively within 22 minutes. This microfluidic device does not meet the same limits of detection compared to ELISA, fluorescence, or ECL immunoassays, but it is still a promising candidate for a commercial POC device for sepsis detection by overcoming other challenges as it rapid and low-cost Yin et al. (2022).

Thermoset plastics have also been used successfully for the fabrication of microfluidic chips as they are highly resistant and insoluble. They are easily manufactured, transparent, and affordable. However, they are not gas-permeable and therefore cannot be used for long-term cell cultures. The most used thermoset plastic in microfluidics is thermoset polyester (TPE) which is more elastic compared to PDMS and TPE valves can be fabricated similarly to those made from PDMS Fiorini et al. (2007). Li, Chang, and Zhang used TPE to develop a chip that was capable of separating haemoglobin from a sample of human blood. This study used TPE over PDMS as it has better optical properties, chemical interness, and mechanical strength, whilst also retaining the ability to be rapidly prototyped Li et al. (2019).

Hydrogels have been proposed as the next-generation material for microfluidics. Hydrogels are 3D water-insoluble hydrophilic polymer networks that contain water-soluble polymers Nie et al. (2020). They can absorb water and can be from either a natural origin, for example, silk, gelatine, or collagen, or from a synthetic origin, such as poly-ethylene(glycol) (PEG), poly(vinyl alcohol) (PVA), and poly(n-iso-propyl acrilamide) (pNIPAA) Ahmed (2015). Hydrogels are attractive materials for microfluidic use due to their compatibility and structural properties that mimic some biological tissues. They are also known to respond to stimuli such as electric and magnetic fields, pH, and temperature, and change state under such conditions Goy et al. (2019). To date, there is no hydrogel microfluidic device specifically designed for early sepsis detection, but a recent study detected cytokines IL-6, IL-8, and MPC-1 using a nano-in-micro smart hydrogel composite microfluidic device which is a promising start for multiplexed hydrogel microfluidics Hsu et al. (2019).

Table 3: Microfluidic Bioreceptor Immobilisation Techniques

Immobilisation technique	Bond stability	pH-dependent	Temperature-dependent	Reversible	Bioreceptor	Microfluidic material	Reference
Physisorption	Low	Yes	Yes	Yes	Antibody	Silicon	Fabri-Faja et al. (2019)
Electrostatic adsorption	High	Yes	No	Yes	Antibody	PMMA, PDMS, silicon, paper, elastomer	Lin et al. (2022)
Chemisorption	High	Yes	Yes	No	Aptamer, antibody, molecularly imprinted polymers (MIPs)	Thermoset plastic, PDMS, silicon, glass	Ma et al. (2020)
Hydrophobic interaction	Medium	Yes	Yes	Yes	DNA	PMMA, PDMA, thermoset plastic	Caneira et al. (2019)
Bioaffinity	Medium	Yes	Yes	Yes	Antibody	Silicon,	Cetin et al.

interaction						PDMS	(2020)
Entrapment, encapsulation	High	Yes	Yes	No	Enzyme	Hydrogel	Dai et al. (2020)

For a microfluidic device to be successful as a POC test it should achieve precise results with minimum effort from the end user. Research has recently focused on incorporating previously discussed materials into fully automated microfluidic devices which use pumps to control fluid flow and eliminate the risk of human error Davis et al. (2021). Microfluidic pumps are responsible for transporting minute quantities of fluid and controlling the flow rate of gases and liquids to achieve sensitive detection of analytes. Pumps used in microfluidics include syringe pumps which can achieve uniform picolitre per minute flow rates which is ideal for microfluidics Zhang et al. (2020), peristaltic pumps which provide variable flow rates Behrens et al. (2020) and, self-priming pumps which have a long-life cycle of up to 20 million dispense cycles Saren et al. (2018). Many recent studies have aimed at developing a microfluidic pump that is low-cost, robust, and requires minimum effort from the end user. Jeon et al., developed a fully automated microfluidic system, consisting of two syringe pumps to monitor immune responses in sepsis by analysing leukocyte activation from 50 μL blood in 25 minutes Jeon et al. (2021).

3.3 Microfluidic Sensing Platform

3.3.1 Electrochemical Sensing and Microfluidics

Microfluidics and electrochemistry have shown great synergy and have been used over colourimetric or optical biosensors due to attractive features such as miniaturisation, high sensitivity and selectivity, low cost, and low sample and reagent requirements. Águeda Molinero-Fernández et al. (2020) Electrochemical microfluidics provides label-free detection, Yu et al. (2006) real-time analysis of assays, Rhouati et al. (2016) and is constructed by the modification of electrode surfaces, for example, silver, gold, or carbon-based conductors with bioreceptors. The analyte concentration is quantitatively detected from changes in the current that cause biological activity at the transducer interface under applied potential or current impulses Ebrahimi et al. (2022). Electrochemical sensors have however been reported to have some drawbacks including low signal-to-noise ratios and fouling of sensor interfaces where contaminated materials interfere with the signal output after prolonged exposure to the sample Durkin et al. (2021). Electrochemical sensors also have repeatability issues, poor shelf life and limited biocompatibility Liu et al. (2019).

Research by Molinero-Fernández et al. used magnetic micromotors paired with an electrochemical microfluidic device to achieve a LOD of 0.54 μg/mL for CRP in under 8 minutes. The device as displayed in Fig. 4 has integrated a thin layer of gold nanoparticles and required a low plasma sample volume of 10 μL. This approach is promising for an electrochemical microfluidic device as a POC test as it is rapid, highly sensitive, and portable Águeda Molinero-Fernández et al. (2020). Another successful study used electrochemiluminescence (ECL) technology, which is a combination of electrochemistry and chemiluminescence with microfluidics to amplify the sensitivity for PCT detection with a LOD of 3.46 fg/mL Song et al. (2022). ECL technology is an electrochemical process where molecules undergo electron-transfer reactions at the electrode surface to create an excited photon-emitted state. ECL detects emitted light and highly sensitive photon detection is made possible due to the development of performance detectors and

sensor systems. Fluorescence remains the favourable light-emission-based detection method for POC devices, but ECL doesn't require an external light source and therefore background signal is reduced with ECL, leading to higher signal-to-noise ratios and lower

LODs compared with fluorescence technology. The ECL signal is only generated at the electrode surface, so ECL is highly localised and time-triggered making it an advantageous technology to use in conjunction with microfluidics Kirschbaum and Baeumner (2015). Nevertheless, challenges associated with the synthesis of new electrode materials with enhanced conductivity and the synthesis of new ECL reagents such as those which originate from small molecules like luminol rather than from fluorescent molecules, require further advancements in the field to maximize the potential of ECL. Qi and Zhang (2019).

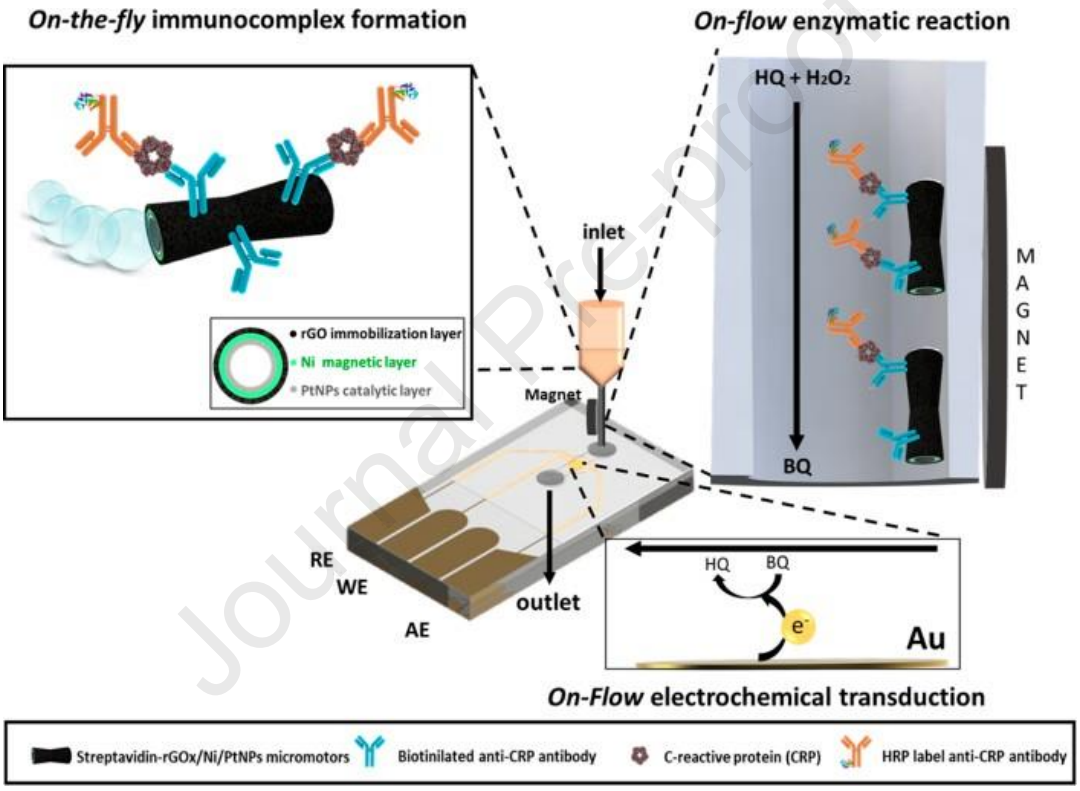


Figure 4: Electrochemical microfluidic technology to test a magnetic micromotor-based immunoassay for CRP determination. Reproduced from ref. Águeda Molinero-Fernández et al. (2020) with permission from ACS Publications, copyright 2020.

3.3.2 Optical Sensing and Microfluidics

Optical sensing techniques that are paired with microfluidic technology for POC sepsis detection are surface plasmon resonance, absorbance, fluorescence, chemiluminescence, interferometers-based techniques, and surface-enhanced Raman spectroscopy. Optical techniques are based on colour change analysis to give a qualitative or semi-qualitative result. This is a disadvantage as it can lead to reduced assay specificity and sensitivity. But quantitative results are possible if the assay is paired with image recognition technology, such as a smartphone with the use of

artificial intelligence (AI) technology.

Surface plasmon resonance (SPR) is a powerful real-time and label-free optical technique used to provide information on the binding kinetics and interaction of binding partners. SPR is the fundamental principle behind many optical biosensors and lab-on-a-chip sensors. The SPR phenomenon occurs when the resonant oscillation of electrons at the interface between positive and negative permittivity material in a particle is stimulated by incident light Vasimalla et al. (2021). A SPR instrument consists of a microfluidic unit to direct fluid flow, a sensing layer which includes a dextran later, a metal film and dielectric medium and an optical detection system. SPR technology has been successfully commercialised, but it still requires bulky instruments and specialist operators. Lab-on-a-chip SPRs are being developed to overcome these challenges and are manufactured using previously discussed materials such as PDMS Lo et al. (2022) and PMMA Yeung et al. (2021). To date, there is no current research that uses an SPR microfluidic platform specifically for sepsis detection. However, Xiao *et al.,* designed a smartphone imaging SPR known as Smart-iSPR, presented in Fig. 5 to measure biomarker affinity interactions and could be optimised specifically for sepsis Xiao et al. (2022).

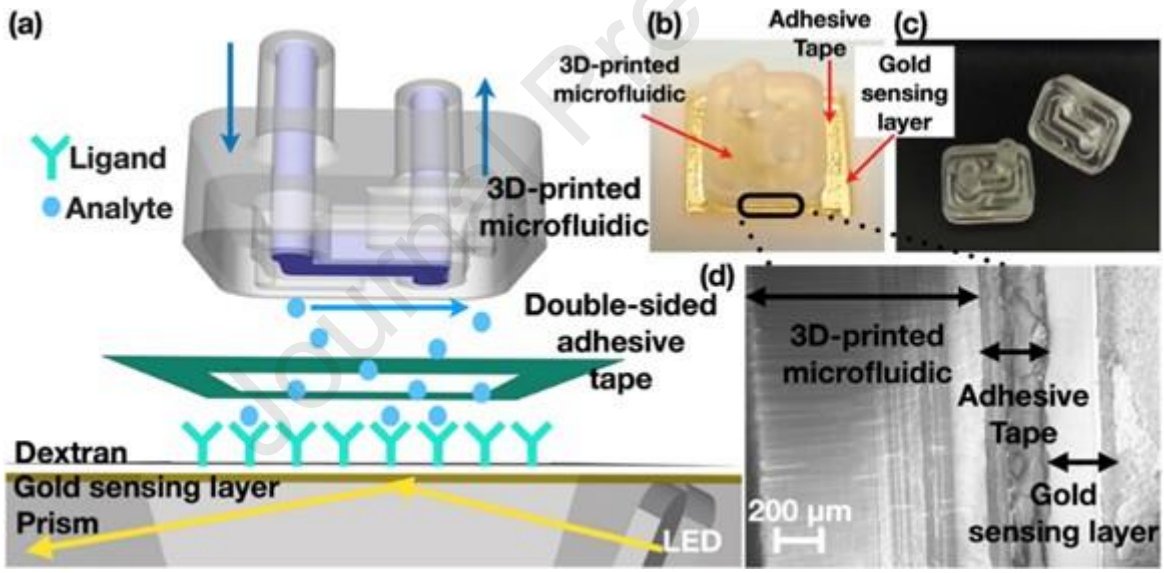


Figure 5: (a) Schematic of the microfluidic chip coupled with SPR sensor (b) Photograph of the microfluidic chip coupled with SPR sensor (c) SEM image of the interface surface between the microfluidic chip and SPR sensor. Reproduced from ref. Xiao et al. (2022) with permission from ELSEVIER, copyright 2022.

One major limitation of SPR, despite its excellent sensitivity, is scaling down the size of the instrument where it is deemed suitable for the routine POC application, at an affordable cost. This challenge is very well addressed by Localized surface resonance (LSPR) instruments as it avoids the use of prism couplers within SPR systems.

LSPR is a phenomenon that is generated by metal nanostructures such as gold nanoparticles absorbing energy caused by light illumination. This causes oscillations of electron charge on the surface of metal nanostructures. LSPR is used in biosensors to detect shifts in the LSPR absorption peak caused by the refractive index change upon

molecule binding or to make use of LSPR to excite fluorescent labels in a sandwich assay Sun et al. (2020). Compared to SPR, LSPR sensor chips can be manufactured at a much more affordable price and do not require strict temperature-controlled environments, meaning that LSPR is more attractive for POC use. The integration of LSPR biosensors within microfluidic devices provides rapid analysis of biomarkers with minimal sample volumes. LSPR allows for real-time measurements of biomarkers by taking advantage of the high surface specificity Hoque et al. (2022). In 2020, Chen et al. developed an automated microfluidic device that incorporated a multiplexed assay for the detection of IgG, TNF-α, and CRP for use as a detection device for sepsis. This style of device utilized LSPR for label-free analyte sensing. LSPR can be easily integrated into a microfluidic device since it is a mechanism that requires only a small area. However, the assay time of 3.5 hours is lengthy in the instance of sepsis and requires a large sample volume of 60 μL Chen et al. (2020). Bhalla et al. used LSPR to achieve a CRP LOD of 2 x 10^{-12} g/mL by exploiting novel silver and titanium nanoislands on a silicon oxide surface which can help to improve product shelf-life due to reducing the oxidation of metals at the nanoscale. Improved product shelf-life within a POC device is advantageous for commercial use Bhalla et al. (2019).

Fluorescence is another optical sensing technique commonly used in microfluidics. Fluorescence detection has been a useful tool for a large range of biomedical applications from drug development and cell imaging to proteomics and disease diagnostics. A recent study demonstrated how a smartphone-imaged microfluidic biodevice could be used to detect CD64 presence on neutrophils from whole human blood in 50 minutes. The microfluidic device was designed to be compatible with a fluorescence microscope Ghonge et al. (2019). Fluorescent labels offer rapid and sensitive quantitative detection of target analytes. However, reagents can be costly which would increase the final cost of the POC device compared to the use of other materials Semeniak et al. (2022). Additionally, fluorescent molecules are prone to quenching effects and therefore making the sensor either short-lived or introducing false hits. SERS has gained a reputation as one of the best optical sensing techniques for label-free detection of biomarkers and is used in many fields such as analytical chemistry, biology, materials science, electrochemistry, forensic science, and environmental science Panneerselvam et al. (2022). Li et al., proposed a CRP and SAA multiplexed lateral flow device based on core-shell AuMBA@mSiO2 Surface Enhanced Ramen Spectroscopy (SERS) nanotags with a LOD of 10 ng/mL in 20 minutes Li et al. (2021). However, SERS has some drawbacks, for instance, (1) SERS need an intimate contact between the enhancing surface and the analyte; (2) the SERS substrates degrade with time resulting in a fall of the sensor signal; (3) limited selectivity of the substrates for a given analyte; (4) limited re-usability of the substrates; and (5) issues associated with optimisation of with homogeneity and reproducibility of the SERS signal within a substrate Mosier-Boss (2017).

3.3.3 Spectrometry Techniques and Microfluidics

Mass spectrometry is commonly used in many different research areas due to its high sensitivity based on its mass-to-charge ratio characteristic and highly attractive label-free process. There are many types of mass spectrometry techniques, many of which are time-consuming, and require large sample volumes, and specialised personnel to use such as liquid chromatography-mass spectrometry (LC/MS) which would not be adequate at the POC. In recent years mass spectrometry techniques have

become more portable and have been used in integration with microfluidics as a label-free biosensing method via various interfaces and ionization methods. The most popular techniques are electrospray ionization (ESI), as demonstrated in Fig. 6 Wu et al. (2018) and matrix-assisted laser desorption ionization (MALDI) paired with microfluidics Shan et al. (2022). ESI pumps force fluid through small channels which are constrained by an electric field that produces a taylor cone that releases charged drops of liquid that are desolvated and entered the mass spectrometry system. MALDI is the most common technique paired with microfluidics for biomarker detection. It offers an automated and extremely high throughput due to its nanosecond timescale, allowing real-time analysis without affecting sample integrity Ha et al. (2021). A summary of microfluidic technology used to detect sepsis biomarkers can be seen in table 4. A main drawback of spectrometry techniques paired with microfluidics is the requirement of additional handling steps, including sampling and sample preparation prior to analysis. Extra handling can compromise sample integrity and throughput. Further work needs to be completed to move towards coupling microfluidics with spectrometry approaches without these additional requirements.

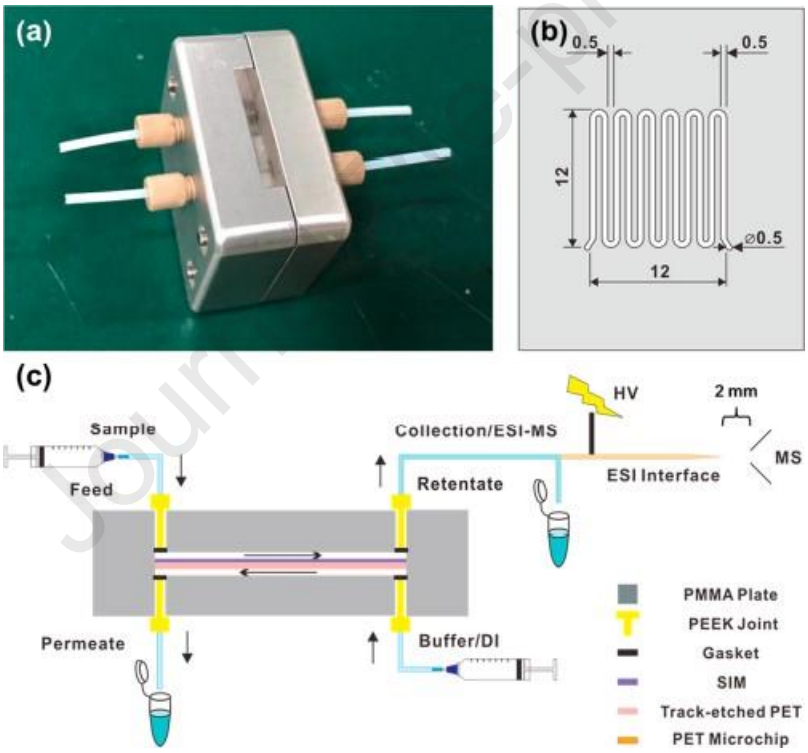


Figure 6: (a) Photograph of a microfluidic device fabricated from a SIM-PET membrane and PMMA channel layers (b) Schematic of the PMMA channel design (c) Schematic of the desalting process and the combination of the membrane-based device with an ESI-MS. Reproduced from ref. Wu et al. (2018) with permission from ACS Publications, copyright 2018.

3.4 Microfluidics to Improve Assay Performance

Microfluidics has been known to improve assay performance, by increasing sensitivity and selectivity. One of the main advantages of microfluidics to enhance

assay specificity is the requirement of low sample volumes. The most common sample type for sepsis biomarker detection at the POC is whole blood from which it is often difficult to use a low sample volume due to the requirement of whole blood filtration. However, microfluidics can overcome this issue as reported by Hassan et al. who developed a microfluidic biochip which only requires 10μL of whole blood to detect CD64 without manual processing Hassan et al. (2017). Other methods require much larger sample volumes, such as the PCR-based Sepsis@Quick test which requires 2 volumes of 1.2mL whole blood Trung et al. (2019). Low sample volume decreases the risk of non-specific binding and background matrix effects, thus increasing specificity.

The ability of microfluidic devices to control assay liquid flow rate can enhance assay sensitivity by firstly ensuring all assay liquid is flown through the system but also have the ability to optimise the assay kinetics. Correct use of running buffers, pressure applied and optimised assay time enhances assay performance by reducing background noise and allowing the correct amount of incubation time for analytes to produce a signal Liu et al. (2021).

Additionally, microfluidics improves multiplex assay performance by reducing interference from different complex sample types, such as whole blood, urine or serum by reducing multiplex biomarker interaction through isolation techniques such as microarrays where each biomarker has its own flow path to prevent non-specific binding He et al. (2020). Also, microfluidics allows precise control of sample volumes, reducing the amount of sample matrix in the assay and providing correct dilution and mixing of the sample to reduce matrix effects. Sample pretreatments such as extraction can also be an option used within microfluidics to allow the separation of analytes from the matrix to minimise interference Su et al. (2021).

4. AI for Microfluidic Technology

AI can allow for the identification of new sepsis biomarkers, specific transducer substrates and materials in microfluidic technology by analyzing large amounts of microfluidic data such as fluid dynamics and biochemical data. Machine learning algorithims can be used to process data and identify patterns and correlations, for example, impedance spectra and electrical signals can help indicate specific transducer substrates Liu et al. (2021).

AI can also help with the analysis of large clinical data sets for risk factor-associated sepsis classification. This can involve analyzing patient demographics, medical histories, and other relevant data to identify risk factors for sepsis, such as age, sex and genetics Wong et al. (2019). By using AI, researchers can also identify new risk factors for sepsis that may not have been previously considered, helping to improve the accuracy of sepsis diagnosis and risk assessment. In addition, AI can be used to generate more personalized sepsis biochips by using patient-specific data to create models that accurately reflect an individual's risk for sepsis. This can involve using data such as genomics and clinical histories to generate a unique model for each patient Ahuja (2019). These personalized models can be used to create sepsis biochips that are tailored to an individual's specific risk profile, allowing for more accurate sepsis diagnosis and personalized treatment plans. Automation of microfluidic devices can be assisted by AI to help improve sensitivity, selectivity and overcome flow-related issues in microfluidics. Automation can also enable the optimisation of microfluidic device design by using simulations to predict the performance of different designs and

determine the best design for a specific application. This can involve optimizing the geometry of microfluidic channels, the selection of materials, and other design parameters to improve the sensitivity, selectivity, and overall performance of microfluidic devices Bhuiyan et al. (2022).

Table 4: Recent Studies using Microfluidic Techniques to Detect Clinical Sepsis Biomarkers

Sepsis biomarker	Microfluidic material	Principle	Signal readout method	Limit of detection (LOD)	Assay time	Sample volume	Dynamic range	Key points	Reference
IL-6	Paper	Paper immunoassay	Colorimetric	0.1 pg/mL	17 mins	2.5 µL	10-0.1 pg/mL	1. Rapid assay time 2. High specificity 3. Low sample volume	Alba-Patino et al. (2020)
CRP and PAB	Paper	Paper immunoassay	Differential pulse voltammetry	5 pg/mL CRP, 10 pg/mL PAB	65 mins	73.3 µL	5 pg/mL-1 µg/mL (CRP), 10 pg/mL - 1 µg/mL (PAB)	1. Multiplexed assay 2. Self-driven capillary action	Sun et al. (2022)
CRP	Paper	ELISA	Colorimetric	10 ng/mL	90 mins	1 µL	1-100 ng/mL (1000-fold diluted blood), 1-100 µg/mL (undiluted blood)	1. Length 5-step procedure 2. Low cost 3. Measured with sheep blood	Verma et al. (2018)
CRP, PCT and IL-6	PDMS	Wave-shaped microfluidic device	Luminescence	0.16 µg/mL CRP, 0.1 ng/mL PCT, 12.5 mg/mL IL-6	22 mins	30 µL	1.25-40 µg/mL (CRP), 0.4-12.8 ng/mL (PCT), 50-1600 pg/mL (IL-6)	1. Multiplex assay 2. Small sample population 3. Rapid assay	Yin et al. (2022)
CRP	PDMS	Micromotor immunoassay	Colorimetric	0.54 µg/mL	8 mins	10 µL	1-100 µg/mL	1. Low sample volume 2. Rapid assay 3. Neonatal sepsis only	Águeda Molinero-Fernández et al. (2020)
PCT	PDMS	ECL immunoassay	ECL	3.46 fg/mL	3.5 hours	20 µL	1-100 µg/mL	1. High sensitivity 2. Portable 3. Automated	Song et al. (2022)
IgG and CRP	PDMS	Plasmonic immunoassay	LSPR	10 ng/mL	3.5 hours	60 µL	n/a	1. Label-free 2. Long assay time 3.	Hoque et al. (2022)
CD64	PDMS	Cell immunocapture	Fluorescence	n/a	50 mins	1	n/a	1. Portable reader 2. Qualitative results 3. lengthy 6-step procedure	Ghonge et al. (2019)
CRP and SAA	Paper	Paper immunoassay	SERS	10 ng/mL	20 mins	90 µL	0.5-1000 ng/mL	1. Rapid assay time 2. High sensitivity 3. Large sample volume	Li et al. (2021)

5. Opportunities and Challenges for Microfluidic Sepsis Technology

We see the following opportunities:

1. Microfluidic technology can detect multiple clinical sepsis biomarkers simultaneously. The capability of a device to detect multiple biomarkers is critical for successful sepsis patient outcomes to aid antibiotic therapy, help to determine the cause of sepsis, and monitor inflammation in recovering patients.
2. Microfluidic technology meets the WHO "ASSURED" guidelines for an ideal POC device Naseri et al. (2022) compared to traditional laboratory-based testing methods. Microfluidic technology for sepsis detection is already commercially available with patented work including a digital microfluidic device which removes white blood cells from whole blood samples to detect bacterial DNA which can help to diagnose sepsis within 6 hours Liu et al. (2019).
3. Microfluidic technology can be paired with many different types of materials and sensing platforms, allowing a range of unique combinations to determine the most suitable POC test for sepsis detection. Future work could focus on pairing a wearable sensor with microfluidics for sepsis detection. This type of sensor could undergo sweat analysis as a non-invasive alternative to whole blood sampling. Wearable sensors are gaining commercial interest and are supported by AI to enhance assay performance and robustness.

We see the following challenges:

1. The interaction between materials used such as plastic, adhesives, and other materials can reduce assay performance. There are many materials, such as those previously discussed being used for microfluidic manufacture, but limitations found with material brittleness, cost, and lack of flexibility have been reported which has delayed devices from being used on a commercial scale, with many devices failing to ever reach the market.
2. The ability to standardize microfluidic technology is limited. Industry-wide standards are required to improve research to market integration and to support guaranteed fitness for use. Standardized microfluidic technology will allow high-quality, safe, and reliable devices to reach the end user.
3. There is a bottleneck between research and a commercial setting due to different expectations. The design of microfluidic technology should always consider the end user. There are a limited number of commercially available microfluidic devices on the market due to challenges faced such as manufacturers not being willing to adapt to change from conventional methods which reduces the incentive to produce such devices. For a microfluidic device to be successful there must be a dramatic cost difference or impeccable performance to outweigh current technologies, which has become a driving force for recent POC diagnostic studies Volpatti and Yetisen (2014).

6. Conclusion

In this review, we have discussed current and novel biomarkers for sepsis detection, multiplexed biomarker assays and microfluidic technology for sepsis detection including microfluidic materials and current microfluidic sensing flatforms. This is followed by

the opportunities and challenges we see for the use of microfluidic technology in relation to sepsis detection. We have concluded that a single biomarker is not specific or sensitive enough to detect sepsis and whilst using a multiplex assay has been a welcomed solution, there is still no optimum combination of biomarkers for sepsis detection. With further research, we strongly feel that an optimum biomarker combination could be achieved. Microfluidic technology is also gaining better multiplexing capabilities, therefore allowing a larger number of biomarkers to be analyzed simultaneously.

Increasing research efforts and progress have been seen in microfluidic technology for sepsis detection, while opportunities and challenges still coexist. Microfluidic technology used within POC devices has been a point of interest for recent studies due to the portable size of microfluidic devices, low manufacturing costs, and the capability of multiplexing biomarkers. Upcoming materials such as thermoset plastics and hydrogels are allowing microfluidic technology to be even more affordable, resistant, and compatible, which is helping to overcome the current bottleneck between researchers and commercial manufacturers.

We see an opportunity for microfluidic technology to act as a POC alternative detection tool for sepsis. Correct assay development combined with a commercially viable microfluidic approach and AI is an exciting prospect for future work. Researchers can use AI to reduce the time and cost required for manual analysis and experimentation and help to advance the field of microfluidics and improve the performance of microfluidic devices.

7. Author Contributions

Z.B. contributed to the writing of the review and N.B. contributed to the discussion and correction of the review.

8. Conflicts of interest

All authors declare no conflict of interest.

References

Águeda Molinero-Fernández, Ángel López, M., Escarpa, A., 2020. An on-chip microfluidic-based electrochemical magneto-immunoassay for the determination of procalcitonin in plasma obtained from sepsis diagnosed preterm neonates. Analyst 145, 5004–5010.

Águeda Molinero-Fernández, Ángel López, M., Escarpa, A., 2020. Electrochemical microfluidic micromotors-based immunoassay for C-reactive protein determination in preterm neonatal samples with sepsis suspicion. Analytical chemistry 92, 5048–5054.

Ahmed, E.M., 2015. Hydrogel: Preparation, characterization, and applications: A review. Journal of advanced research 6, 105–121.

Ahuja, A.S., 2019. The impact of artificial intelligence in medicine on the future role of the physician. PeerJ 7, e7702.

Akyazi, T., Basabe-Desmonts, L., Benito-Lopez, F., 2018. Review on microfluidic paper-based analytical devices towards commercialisation. Analytica chimica acta 1001, 1–17.

Alba-Patino, A., Russell, S.M., Borges, M., Pazos-Pérez, N., Álvarez Puebla, R.A., de la Rica, R., 2020. Nanoparticle-based mobile biosensors for the rapid detection of sepsis biomarkers in whole blood. Nanoscale Advances 2, 1253–1260.

Alba-Patino, A., Russell, S.M., Borges, M., Pazos-Pérez, N., Álvarez Puebla, R.A., de la Rica, R., 2020. Nanoparticle-based mobile biosensors for the rapid detection of sepsis biomarkers in whole blood. Nanoscale Advances 2, 1253–1260.

An integrated microfluidic system for early detection of sepsis-inducing bacteria. Lab on a Chip 21,

113–121.
Ariati, R., Sales, F., Souza, A., Lima, R.A., Ribeiro, J., 2021. Polydimethylsiloxane composites characterization and its applications: A review. Polymers 13, 4258.
Barr, B., Nieman, N.M., 2022. Serum amyloid A as an aid in diagnosing sepsis in equine neonates. Equine Veterinary Journal 54, 922–926.
Behrens, M.R., Fuller, H.C., Swist, E.R., Wu, J., Islam, M., Long, Z., Ruder, W.C., Steward, R., 2020. Open- source, 3D-printed peristaltic pumps for small volume point-of-care liquid handling. Scientific reports 10, 1–10.
Bengnér, J., Quttineh, M., Gäddlin, P.O., Salomonsson, K., Faresjö, M., 2021. Serum amyloid A-A prime candidate for the identification of neonatal sepsis. Clinical Immunology 229, 108787.
Bhalla, N., Jamshaid, A., Leung, M.H., Ishizu, N., Shen, A.Q., 2019. Electrical contact of metals at the nanoscale overcomes the oxidative susceptibility of silver-based nanobiosensors. ACS Applied Nano Materials 2, 2064–2075.
Bhuiyan, N.H., Hong, J.H., Uddin, M.J., Shim, J.S., 2022. Artificial intelligence-controlled microfluidic device for fluid automation and bubble removal of immunoassay operated by a smartphone. Analytical Chemistry 94, 3872–3880.
Caneira, C.R., Soares, R.R., Pinto, I.F., Mueller-Landau, H.S., Azevedo, A.M., Chu, V., Conde, J.P., 2019. Development of a rapid bead-based microfluidic platform for DNA hybridization using single-and multi- mode interactions for probe immobilization. Sensors and Actuators B: Chemical 286, 328–336.
Cetin, D., Okan, M., Bat, E., Kulah, H., 2020. A comparative study on EpCAM antibody immobilization on gold surfaces and microfluidic channels for the detection of circulating tumor cells. Colloids and Surfaces B: Biointerfaces 188, 110808.
Cetinkaya, M., Özkan, H., Köksal, N., Celebi, S., Hacğlu, M., 2009. Comparison of serum amyloid A concentrations with those of C-reactive protein and procalcitonin in diagnosis and follow-up of neonatal sepsis in premature infants. Journal of Perinatology 29, 225–231.
Chen, J.S., Chen, P.F., Lin, H.T.H., Huang, N.T., 2020. A Localized surface plasmon resonance (LSPR) sensor integrated automated microfluidic system for multiplex inflammatory biomarker detection. Analyst 145, 7654–7661.
Claxton, A., Papafilippou, L., Hadjidemetriou, M., Kostarelos, K., Dark, P., 2020. The challenge of recognising sepsis: future nanotechnology solutions. Journal of the Intensive Care Society 21, 241–246.
Cohen, J., Vincent, J.L., Adhikari, N.K., Machado, F.R., Angus, D.C., Calandra, T., Jaton, K., Giulieri, S., Delaloye, J., Opal, S., 2015. Sepsis: a roadmap for future research. The Lancet infectious diseases 15, 581–614.
Cong, S., Ma, T., Di, X., Tian, C., Zhao, M., Wang, K., 2021. Diagnostic value of neutrophil CD64, procalcitonin, and interleukin-6 in sepsis: A meta-analysis. BMC Infectious Diseases 21, 1–17.
da Gomes Cunha, D.M., da Silva, G.G., Hamasaki, M.Y., 2020. New biomarkers of sepsis with clinical relevance.
Dai, J., Zhang, H., Huang, C., Chen, Z., Han, A., 2020. A gel-based separation-free point-of-care device for whole blood glucose detection. Analytical Chemistry 92, 16122–16129.
Damas, P., Ledoux, D., Nys, M., Vrindts, Y., Groote, D.D., Franchimont, P., Lamy, M., 1992. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Annals of surgery 215, 356.
Davis, J.J., Padalino, M., Kaplitz, A.S., Murray, G., Foster, S.W., Maturano, J., Grinias, J.P., 2021. Utility of low-cost, miniaturized peristaltic and Venturi pumps in droplet microfluidics. Analytica Chimica Acta 1151, 338230.
den Brand, M.V., den Dungen, F.A.V., Bos, M.P., Weissenbruch, M.M.V., Furth, A.V., Lange, A.D., Rubenjan, A., Peters, R.P., Savelkoul, P.H., 2018. Evaluation of a real-time PCR assay for detection and quantification of bacterial DNA directly in blood of preterm neonates with suspected late-onset sepsis. Critical Care 22, 1–10.
Dimoula, A., Pradier, O., Kassengera, Z., Dalcomune, D., Turkan, H., Vincent, J.L., 2013. Serial Determinations of Neutrophil CD64 Expression for the Diagnosis and Monitoring of Sepsis in Critically Ill Patients. Clinical Infectious Diseases 58, 820–829.
Drain, P.K., Hyle, E.P., Noubary, F., Freedberg, K.A., Wilson, D., Bishai, W.R., Rodriguez, W., Bassett, I.V., 2014. Diagnostic point-of-care tests in resource-limited settings. The Lancet infectious diseases 14, 239–249.
Du, J., Li, L., Dou, Y., Li, P., Chen, R., Liu, H., 2014. Diagnostic utility of neutrophil CD64 as a marker for early-onset sepsis in preterm neonates. PloS one 9, e102647.

Durkin, T.J., Barua, B., Savagatrup, S., 2021. Rapid detection of sepsis: Recent advances in biomarker sensing platforms. ACS omega 6, 31390–31395.

Durkin, T.J., Barua, B., Savagatrup, S., 2021. Rapid detection of sepsis: Recent advances in biomarker sensing platforms. ACS omega 6, 31390–31395.

Ebrahimi, G., Pakchin, P.S., Shamloo, A., Mota, A., de la Guardia, M., Omidian, H., Omidi, Y., 2022. Label- free electrochemical microfluidic biosensors: futuristic point-of-care analytical devices for monitoring diseases. Microchimica Acta 189, 1–18.

Eichberger, J., Resch, B., 2022. Reliability of Interleukin-6 Alone and in Combination for Diagnosis of Early Onset Neonatal Sepsis: Systematic Review. Frontiers in Pediatrics 10.

Eichberger, J., Resch, E., Resch, B., 2022. Diagnosis of Neonatal Sepsis: The Role of Inflammatory Markers. Frontiers in Pediatrics 10.

Eschborn, S., Weitkamp, J.H., 2019. Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. Journal of Perinatology 39, 893–903.

Fabri-Faja, N., Calvo-Lozano, O., Dey, P., Terborg, R.A., Estevez, M.C., Belushkin, A., Yesilköy, F., Duempelmann, L., Altug, H., Pruneri, V., 2019. Early sepsis diagnosis via protein and miRNA biomarkers using a novel point-of-care photonic biosensor. Analytica chimica acta 1077, 232–242.

Fallahi, H., Zhang, J., Phan, H.P., Nguyen, N.T., 2019. Flexible microfluidics: Fundamentals, recent developments, and applications. Micromachines 10, 830.

Fang, Y.L., Wang, C.H., Chen, Y.S., Chien, C.C., Kuo, F.C., You, H.L., Lee, M.S., Lee, G.B., 2021. An integrated microfluidic system for early detection of sepsis-inducing bacteria. Lab on a Chip 21, 113–121. Fang, Y.L., Wang, C.H., Chen, Y.S., Chien, C.C., Kuo, F.C., You, H.L., Lee, M.S., Lee, G.B., 2021.

Fiorini, G.S., Yim, M., Jeffries, G.D., Schiro, P.G., Mutch, S.A., Lorenz, R.M., Chiu, D.T., 2007. Fabrication improvements for thermoset polyester (TPE) microfluidic devices. Lab on a Chip 7, 923–926.

Ghazal, P., Rodrigues, P.R., Chakraborty, M., Oruganti, S., Woolley, T.E., 2022. Challenging molecular dogmas in human sepsis using mathematical reasoning. eBioMedicine 80, 104031.

Ghonge, T., Koydemir, H.C., Valera, E., Berger, J., Garcia, C., Nawar, N., Tiao, J., Damhorst, G.L., Ganguli, A., Hassan, U., 2019. Smartphone-imaged microfluidic biochip for measuring CD64 expression from whole blood. Analyst 144, 3925–3935.

Goy, C.B., Chaile, R.E., Madrid, R.E., 2019. Microfluidics and hydrogel: A powerful combination. Reactive and Functional Polymers 145, 104314.

Guo, W., Hansson, J., van der Wijngaart, W., 2020. Synthetic paper separates plasma from whole blood with low protein loss. Analytical chemistry 92, 6194–6199.

Ha, N.S., de Raad, M., Golini, A., Petzold, C.J., Northen, T.R., 2021. Faster, better, and cheaper: harnessing microfluidics and mass spectrometry for biotechnology. RSC Chemical Biology 2, 1331–1351.

Hassan, U., Ghonge, T., Jr, B.R., Patel, M., Rappleye, M., Taneja, I., Tanna, A., Healey, R., Manusry, N., Price, Z., 2017. A point-of-care microfluidic biochip for quantification of CD64 expression from whole blood for sepsis stratification. Nature communications 8, 1–12.

Hassanpour-Tamrin, S., Sanati-Nezhad, A., Sen, A., 2021. A simple and low-cost approach for irreversible bonding of polymethylmethacrylate and polydimethylsiloxane at room temperature for high-pressure hybrid microfluidics. Scientific Reports 11, 1–12.

He, Z., Huffman, J., Curtin, K., Garner, K.L., Bowdridge, E.C., Li, X., Nurkiewicz, T.R., Li, P., 2020. Composable microfluidic plates (cPlate): A simple and scalable fluid manipulation system for multiplexed enzyme-linked immunosorbent assay (ELISA). Analytical chemistry 93, 1489–1497.

Hirao, Y., Kanda, T., Aso, Y., Mitsuhashi, M., Kobayashi, I., 2000. Interleukin-8—an early marker for bacterial infection. Laboratory medicine 31, 39–44.

Hoque, S.Z., Somasundaram, L., Samy, R.A., Dawane, A., Sen, A.K., 2022. Localized Surface Plasmon Resonance Sensors for Biomarker Detection with On-Chip Microfluidic Devices in Point-of-Care Diagnostics.

Hsu, M.N., Wei, S.C., Phan, D.T., Zhang, Y., Chen, C.H., 2019. Nano-in-Micro Smart Hydrogel Composite for a Rapid Sensitive Immunoassay. Advanced Healthcare Materials 8, 1801277.

Hung, S.K., Lan, H.M., Han, S.T., Wu, C.C., Chen, K.F., 2020. Current evidence and limitation of biomarkers for detecting sepsis and systemic infection. Biomedicines 8, 494.
Infection, disease & health 24, 44–48.

Jeon, H., Lee, D.H., Jundi, B., Pinilla-Vera, M., Baron, R.M., Levy, B.D., Voldman, J., Han, J., 2021. Fully automated, sample-to-answer leukocyte functional assessment platform for continuous sepsis

monitoring via microliters of blood. ACS sensors 6, 2747–2756.
Junior, L.K.O., Carmona, F., Aragon, D.C., Gonçalves-Ferri, W.A., 2021. Evaluation of urine output, lactate levels and lactate clearance in the transitional period in very low birth weight preterm infants. European Journal of Pediatrics 180, 91–97.
Kirschbaum, S.E., Baeumner, A.J., 2015. A review of electrochemiluminescence (ECL) in and for microfluidic analytical devices. Analytical and bioanalytical chemistry 407, 3911–3926.
Lenski, M., Huge, V., Briegel, J., Tonn, J.C., Schichor, C., Thon, N., 2017. Interleukin 6 in the cerebrospinal fluid as a biomarker for onset of vasospasm and ventriculitis after severe subarachnoid hemorrhage. World Neurosurgery 99, 132–139.
Li, D., Zhang, Z., Chang, H., 2019. Chip-based nanoflow liquid chromatography for on-chip detection of glycated haemoglobin levels. Sensors and Actuators B: Chemical 291, 433–440.
Li, H., Steckl, A.J., 2018. Paper microfluidics for point-of-care blood-based analysis and diagnostics. Ana- lytical chemistry 91, 352–371.
Li, Y., Liu, X., Guo, J., Zhang, Y., Guo, J., Wu, X., Wang, B., Ma, X., 2021. Simultaneous detection of inflammatory biomarkers by SERS nanotag-based lateral flow assay with portable cloud Raman spec- trometer. Nanomaterials 11, 1496.
Lin, D., Li, B., Fu, L., Qi, J., Xia, C., Zhang, Y., Chen, J., Choo, J., Chen, L., 2022. A novel polymer-based nitrocellulose platform for implementing a multiplexed microfluidic paper-based enzyme-linked immunosorbent assay. Microsystems & Nanoengineering 8, 1–10.
Liu, D., Shen, H., Zhang, Y., Shen, D., Zhu, M., Song, Y., Zhu, Z., Yang, C., 2021. A microfluidic-integrated lateral flow recombinase polymerase amplification (MI-IF-RPA) assay for rapid COVID-19 detection. Lab on a Chip 21, 2019–2026.
Liu, J., Jiang, X., Zhang, R., Zhang, Y., Wu, L., Lu, W., Li, J., Li, Y., Zhang, H., 2019. MXene-enabled electrochemical microfluidic biosensor: applications toward multicomponent continuous monitoring in whole blood. Advanced Functional Materials 29, 1807326.
Liu, J., Li, S., Cai, C., Xu, Y., Jiang, Y., Chen, Z., 2018. Cerebrospinal fluid chemokine patterns in children with enterovirus 71-related encephalitis. Scientific reports 8, 1–9.
Liu, L., Bi, M., Wang, Y., Liu, J., Jiang, X., Xu, Z., Zhang, X., 2021. Artificial intelligence-powered microfluidics for nanomedicine and materials synthesis. Nanoscale 13, 19352–19366.
Liu, Y., WALTHER-ANTONION, M.R., CHIA, N., Nelson, H., 2019. Point-of-care digital microfluidics for sepsis diagnosis.
Lo, S.C., Li, S.S., Yang, W.F., Wu, K.C., Wei, P.K., Sheen, H.J., Fan, Y.J., 2022. A Co-Printed Nanoslit Surface Plasmon Resonance Structure in Microfluidic Device for LMP-1 Detection. Biosensors 12, 653.
Loots, F.J., Arpots, R., van den Berg, R., Hopstaken, R.M., Giesen, P., Smits, M., 2017. Recognition of sepsis in primary care: a survey among GPs. BJGP open 1.
Ma, W., Liu, L., Xu, Y., Wang, L., Chen, L., Yan, S., Shui, L., Wang, Z., Li, S., 2020. A highly efficient pre-concentration route for rapid and sensitive detection of endotoxin based on an electrochemical biosensor. Analyst 145, 4204–4211.
McGregor, C., 2014. Improving time to antibiotics and implementing the" Sepsis 6". BMJ Open Quality 2, u202548–w1443.
Min, J., Nothing, M., Coble, B., Zheng, H., Park, J., Im, H., Weber, G.F., Castro, C.M., Swirski, F.K., Weissleder, R., 2018. Integrated biosensor for rapid and point-of-care sepsis diagnosis. Acs Nano 12, 3378–3384.
Morris, E., McCartney, D., Lasserson, D., den Bruel, A.V., Fisher, R., Hayward, G., 2017. Point-of-care lactate testing for sepsis at presentation to health care: a systematic review of patient outcomes. British Journal of General Practice 67, e859–e870.
Mosier-Boss, P.A., 2017. Review of SERS substrates for chemical sensing. Nanomaterials 7, 142.
Mou, L., Jiang, X., 2017. Materials for microfluidic immunoassays: a review. Advanced healthcare materials 6, 1601403.
Mussap, M., Degrandi, R., Cataldi, L., Fanos, V., Plebani, M., 2007. Biochemical markers for the early assessment of neonatal sepsis: the role of procalcitonin. Journal of Chemotherapy 19, 35–38.
Mysler, E., Psioni, C., Tate, P., Tate, G., 2004. Influence of corticosteroids on C-reactive protein in patients with rheumatoid arthritis. Arthritis Res Ther 6, 1–41.
Naseri, M., Ziora, Z.M., Simon, G.P., Batchelor, W., 2022. ASSURED-compliant point-of-care diagnostics for the detection of human viral infections.
Ng, P.C., 2004. Diagnostic markers of infection in neonates. Archives of Disease in Childhood-Fetal and Neonatal Edition 89, 229–235.

Nguyen, H.B., Loomba, M., Yang, J.J., Jacobsen, G., Shah, K., Otero, R.M., Suarez, A., Parekh, H., Jaehne, A., Rivers, E.P., 2010. Early lactate clearance is associated with biomarkers of inflammation, coagulation, apoptosis, organ dysfunction and mortality in severe sepsis and septic shock. Journal of inflammation 7, 1–11.

Nie, J., Fu, J., He, Y., 2020. Hydrogels: The Next Generation Body Materials for Microfluidic Chips? Small 16, 2003797.

Oh, J., Joung, H.A., Han, H.S., Kim, J.K., Kim, M.G., 2018. A hook effect-free immunochromatographic assay (HEF-ICA) for measuring the C-reactive protein concentration in one drop of human serum. Theranostics 8, 3189.

Ozdemir, S.A., Colak, R., Ergon, E.Y., Calkavur, S., 2020. Diagnostic value of urine strem-1 and urine c-reactive protein for infants with late onset neonatal sepsis. Journal of Pediatric Infectious Diseases 15, 072–078.

Panneerselvam, R., Sadat, H., Höhn, E.M., Das, A., Noothalapati, H., Belder, D., 2022. Microfluidics and surface-enhanced Raman spectroscopy, a win-win combination? Lab on a Chip 22, 665–682.

Pant, A., Mackraj, I., Govender, T., 2021. Advances in sepsis diagnosis and management: a paradigm shift towards nanotechnology. Journal of Biomedical Science 28, 1–30.

Papafilippou, L., Claxton, A., Dark, P., Kostarelos, K., Hadjidemetriou, M., 2020. Protein corona fingerprinting to differentiate sepsis from non-infectious systemic inflammation. Nanoscale 12, 10240–10253.

Patel, K., McElvania, E., 2019. Diagnostic challenges and laboratory considerations for pediatric sepsis.

Pierrakos, C., Vincent, J.L., 2010. Sepsis biomarkers: a review. Critical care 14, 1–18.

PloS one 10, e01271.

Povoa, P., Coelho, L., Almeida, E., Fernandes, A., Mealha, R., Moreira, P., Sabino, H., 2005. C-reactive protein as a marker of infection in critically ill patients. Clinical microbiology and infection11, 101–108.

Qi, H., Zhang, C., 2019. Electrogenerated chemiluminescence biosensing. Analytical Chemistry 92, 524–534.

Raveendran, A.V., Kumar, A., Gangadharan, S., 2019. Biomarkers and newer laboratory investigations in the diagnosis of sepsis. Journal of the Royal College of Physicians of Edinburgh 49, 207–216.

Rey, E.G., O'Dell, D., Mehta, S., Erickson, D., 2017. Mitigating the hook effect in lateral flow sandwich immunoassays using real-time reaction kinetics. Analytical chemistry 89, 5095–5100.

Rhouati, A., Catanante, G., Nunes, G., Hayat, A., Marty, J.L., 2016. Label-free aptasensors for the detection of mycotoxins. Sensors 16, 2178.

Ross, G.M., Filippini, D., Nielen, M.W., Salentijn, G.I., 2020. Unraveling the hook effect: a comprehensive study of high antigen concentration effects in sandwich lateral flow immunoassays. Analytical chemistry 92, 15587–15595.

Saren, A., Smith, A.R., Ullakko, K., 2018. Integratable magnetic shape memory micropump for high-pressure, precision microfluidic applications. Microfluidics and Nanofluidics 22, 1–10.

Semeniak, D., Cruz, D.F., Chilkoti, A., Mikkelsen, M.H., 2022. Plasmonic Fluorescence Enhancement in Diagnostics for Clinical Tests at Point-of-Care: A Review of Recent Technologies. Advanced Materials, 2107986.

Shan, L., Gao, H., Zhang, J., Li, W., Su, Y., Guo, Y., 2022. Plasma and serum exosome markers analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry coupled with electron multiplier. Talanta, 123560.

Shiferaw, B., Bekele, E., Kumar, K., Boutin, A., Frieri, M., 2016. The role of procalcitonin as a biomarker in sepsis. J Infect Dis Epidemiol 2, 10–23937.

Shim, B.S., Yoon, Y.H., Kim, J.Y., Cho, Y.D., Park, S.J., Lee, E.S., Choi, S.H., 2019. Clinical value of whole blood procalcitonin using point of care testing, quick sequential organ failure assessment score, C-reactive protein and lactate in emergency department patients with suspected infection. Journal of Clinical Medicine 8, 833.

Sliding-strip microfluidic device enables ELISA on paper. Biosensors and Bioelectronics 99, 77–84.

Song, X., Yu, S., Zhao, L., Guo, Y., Ren, X., Ma, H., Wang, S., Luo, C., Li, Y., Wei, Q., 2022. Efficient ABEI- Dissolved O2-Ce (III, IV)-MOF Ternary Electrochemiluminescent System Combined with Self-Assembled Microfluidic Chips for Bioanalysis. Analytical Chemistry 94, 9363–9371.

Su, W., Liang, D., Tan, M., 2021. Microfluidic strategies for sample separation and rapid detection of food allergens. Trends in Food Science & Technology 110, 213–225.

Sun, L.L., Leo, Y.S., Zhou, X., Ng, W., Wong, T.I., Deng, J., 2020. Localized surface plasmon resonance-based point-of-care system for sepsis diagnosis. Materials Science for Energy Technologies 3, 274–

281.
Sun, S., Luo, J., Zhu, Y., Kong, F., Mao, G., Ming, T., Xing, Y., Liu, J., Dai, Y., Yan, S., 2022. Multi-functional self-driven origami paper-based integrated microfluidic chip to detect CRP and PAB in whole blood. Biosensors and Bioelectronics 208, 114225.
Sweeney, T.E., Liesenfeld, O., May, L., 2019. Diagnosis of bacterial sepsis: why are tests for bacteremia not sufficient? Expert Review of Molecular Diagnostics 19, 959–962.
Tanak, A.S., Muthukumar, S., Krishnan, S., Schully, K.L., Clark, D.V., Prasad, S., 2021. Multiplexed cytokine detection using electrochemical point-of-care sensing device towards rapid sepsis endotyping. Biosensors and Bioelectronics 171, 112726.
Tang, Z., Qin, D., Tao, M., Lv, K., Chen, S., Zhu, X., Li, X., Chen, T., Zhang, M., Zhong, M., 2018. Examining the utility of the CD64 index compared with other conventional indices for early diagnosis of neonatal infection. Scientific reports 8, 1–7.
Teggert, A., Datta, H., Ali, Z., 2020. Biomarkers for point-of-care diagnosis of sepsis. Micromachines 11, 286.
The journal of applied laboratory medicine 3, 587–600.
Tro`, R., Gruarin, M., Foglia, A., Agnoli, C., Dondi, F., Giunti, M., 2017. Serum amyloid A in the diagnosis of feline sepsis. Journal of Veterinary Diagnostic Investigation 29, 856–859.
Trung, N.T., Thau, N.S., Bang, M.H., Song, L.H., 2019. PCR-based Sepsis@ Quick test is superior in comparison with blood culture for identification of sepsis-causative pathogens. Scientific reports 9, 1–7.
Vasimalla, Y., Pradhan, H., Pandya, R., 2021. Recent progress in surface plasmon resonance based sensors: A comprehensive review. Heliyon 7, e06321.
Verma, M.S., Tsaloglou, M.N., Sisley, T., Christodouleas, D., Chen, A., Milette, J., Whitesides, G.M., 2018.
Vignon, P., Laterre, P.F., Daix, T., François, B., 2020. New Agents in Development for Sepsis: Any Reason for Hope? Drugs 80, 1751–1761.
Vincent, J.L., 2016. The clinical challenge of sepsis identification and monitoring. PLoS medicine 13, e1002022.
Vo, T.N.A., Chen, P.C., 2022. Maximizing interfacial bonding strength between PDMS and PMMA sub- strates for manufacturing hybrid microfluidic devices withstanding extremely high flow rate and high operation pressure. Sensors and Actuators A: Physical 334, 113330.
Volpatti, L.R., Yetisen, A.K., 2014. Commercialization of microfluidic devices. Trends in biotechnology 32, 347–350.
Weidhase, L., Wellhöfer, D., Schulze, G., Kaiser, T., Drogies, T., Wurst, U., Petros, S., 2019. Is Interleukin-6 a better predictor of successful antibiotic therapy than procalcitonin and C-reactive protein? A single centre study in critically ill adults. BMC Infectious Diseases 19, 1–7.
Wong, Z.S., Zhou, J., Zhang, Q., 2019. Artificial intelligence for infectious disease big data analytics.
Wu, W., Zhang, D., Chen, K., Zhou, P., Zhao, M., Qiao, L., Su, B., 2018. Highly efficient desalting by silica isoporous membrane-based microfluidic chip for electrospray ionization mass spectrometry. Analytical chemistry 90, 14395–14401.
Xiao, C., Eriksson, J., Suska, A., Filippini, D., Mak, W.C., 2022. Print-and-stick unibody microfluidics coupled surface plasmon resonance (SPR) chip for smartphone imaging SPR (Smart-iSRP). Analytica Chimica Acta 1201, 339606.
Yang, R.Z., Lee, M.J., Hu, H., Pollin, T.I., Ryan, A.S., Nicklas, B.J., Snitker, S., Horenstein, R.B., Hull, K., Goldberg, N.H., 2006. Acute-phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS medicine 3, e287.
Yeh, C.F., Wu, C.C., Liu, S.H., Chen, K.F., 2019. Comparison of the accuracy of neutrophil CD64, procalcitonin, and C-reactive protein for sepsis identification: a systematic review and meta-analysis. Annals of intensive care 9, 1–12.
Yeung, W.K., Lo, S.C., Wang, S.H., Wei, P.K., Cheng, J.Y., 2021. Dispersion-enhancing surface treatment of AuNPs for a reduced probe loading and detection limit using t-SPR detection. Analyst 146, 5584–5591.
Yin, B.F., Wan, X.H., Yang, M.Z., Qian, C.C., Sohan, A.S.M., 2022. Wave-shaped microfluidic chip assisted point-of-care testing for accurate and rapid diagnosis of infections. Military Medical Research 9, 1–13.
Yu, X., Xu, D., Cheng, Q., 2006. Label-free detection methods for protein microarrays. Proteomics 6, 5493–5503.
Yuk, S.A., Sanchez-Rodriguez, D.A., Tsifansky, M.D., Yeo, Y., 2018. Recent advances in nanomedicine

for sepsis treatment. Therapeutic delivery 9, 435–450.
Zarogoulidis, P., Yarmus, L., Darwiche, K., Walter, R.F.H., dong Huang, H., Li, Z., Zaric, B., Tsakiridis, K., Zarogoulidis, K., 2013. Interleukin-6 cytokine: a multifunctional glycoprotein for cancer. Immunome research 9 62, 16535.
Zhang, X., Xia, K., Ji, A., 2020. A portable plug-and-play syringe pump using passive valves for microfluidic applications. Sensors and Actuators B: Chemical 304, 127331.
Zhou, M., Cheng, S., Yu, J., Lu, Q., 2015. Interleukin-8 for diagnosis of neonatal sepsis: a meta-analysis.

Highlights

- Demonstrate a range of clinical biomarkers available for the detection of sepsis
- Discusses several approaches based on microfluidics used for point of care diagnosis of sepsis
- Opportunities and challenges in detection of sepsis for lab-to-fab translation
- Need to develop multiplexed systems for reliable detection of sepsis