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Vibrational spectroscopic analysis of blood for diagnosis of infections and sepsis: a review of requirements for a rapid diagnostic test

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Infections and sepsis represent a growing global burden. There is a widespread clinical need for a rapid, high-throughput and sensitive technique for the diagnosis of infections and detection of invading pathogens and the presence of sepsis. Current diagnostic methods primarily consist of laboratory-based haematology, biochemistry and microbiology that are time consuming, labour- and resource-intensive, and prone to both false positive and false negative results. Current methods are insufficient for the increasing demands on healthcare systems, causing delays in diagnosis and initiation of treatment, due to the intrinsic time delay in sample preparation, measurement, and analysis. Vibrational spectroscopic techniques can overcome these limitations by providing a rapid, label-free and low-cost method for blood analysis, with limited sample preparation required, potentially revolutionising clinical diagnostics by producing actionable results that enable early diagnosis, leading to improved patient outcomes. This review will discuss the challenges associated with the diagnosis of infections and sepsis, primarily within the UK healthcare system. We will consider the clinical potential of spectroscopic point-of-care technologies to enable blood analysis in the primary-care setting.

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1. Introduction

1.1 Infections

An infection can be defined as the "pathologic process caused by the invasion of normally sterile tissue or fluid or body cavity by pathogenic or potentially pathogenic microorganisms".[1] Infection can result from bacterial, viral, yeast or fungal microbial invasion. Despite advances in the delivery of more effective and efficient healthcare, infections are becoming increasingly common.[2] In part this is due to more comprehensive reporting, but also to factors such as aging populations, existing co-morbidities and increasing use of invasive procedures.[3] In the last 40 years the mortality rate due to infectious diseases in the UK has seen an overall decline due to improvements in healthcare and lifestyle,[4] but still constitute ~7% of recorded deaths.[5]

Infections are particularly common in intensive care units (ICUs), immunosuppressed, and in elderly and critically ill patients. There are different factors contribution to infection susceptibility, such as age or impaired immune system due to disease or drugs. Table 1 lists the most significant factors that increase the risk of significant infection. Hospital acquired infections (HAIs) are the most common adverse effect associated with the delivery of healthcare in developed countries, primarily due to the proximity of unwell and high-risk patients along with the likelihood of patients undergoing invasive procedures. Over 300 000 patients annually (~6%) are affected with HAIs in England, at a costs of around £1 billion to the NHS:[6,7] predominately respiratory tract (22.8%), urinary tract (17.2%), surgical site (15.7%) infections and clinical sepsis (10.5%).[8] The incidence of HAIs is 3.7–11.6% in developed countries,[9] rising to 14–31% in developing countries.[10]

Overall, in the last 40 years, there has been a general trend in the increase of the life expectancies of both males (~8.3 years) and females (~6.1 years) in the UK.[4] However, a longer life expectancy does not necessarily reflect a healthier population. Pre-existing co-morbidities are particularly common in the elderly, for example, with 64.9% of people over the age of 65 in Scotland having two or more chronic medical conditions.[11] Co-morbidities alone are not sufficient to increase susceptibility to infection but may involve other factors such as recurrent

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Table 1 Factors increasing risk of patients developing infection or sepsis

Risk	Factors increasing risk of infection
Immunocompromised patients	• Underlying malignancy – neutropenia, tumour obstruction, curative treatment, nutritional factors and use of invasive devices[93] • Anticancer or immunomodulatory therapies suppress the normal function of the immune system. Patient may be neutropenic[93] • The bone marrow is unable to maintain production of white blood cells at required rate[21]
Under 1 year of age	• Premature[94] • Low birth weight[35] • Traumatic delivery[95] • Maternal infection[96] • No vaccinations[97] • Immune system still developing[21]
Over 75 years of age	Extracted from ref. 12: • Limited mobility causing skin breakdown and ulcers • Loss of coordination resulting in injury and falls • Poor urinary bladder emptying • Increased incidence of obstructions (gallstones, and urinary tract stones) • Age-related diseases including dementia • Immunosenescence – poor response to vaccinations and increasing incidence of infections
Surgery/invasive procedure in past 6 weeks	• Prolonged operation[98] • Surgical site[99] • Surgery complexity[100] • Patient factors – existing comorbidities, age, frailty[98] • Complications encountered in surgery[100]
Pregnancy	• Immune alterations with advancing pregnancy[101] • Pathophysiological changes (for example decrease in respiratory volume and increase in heart rate)[102] • Aerobic and anaerobic bacteria in vagina and endocervical canal[102]
Breach of skin integrity (cuts, burns, blisters for example)	• Skin is the body's external first line of defence • When the skin is broken, microorganisms can enter the wound and cause an infection[103]
Intravenous drug users (IDU)	• Sharing of needles and syringes[104] • Unsterile injection practices[104] • Contaminated drugs can cause spore-forming infections[105] • Existing diagnosis of HIV, hepatitis C, hepatitis B[106]
Invasive devices (urinary catheter, PICC line for example)	• Contamination of device during insertion[107] • Inefficient cleaning and sterilisation of insertion site[108] • Unnatural opening into the body can allow bacteria and fungi to enter[108] • Lack of frequency of line replacement[107]

hospitalizations, medications and the immune system senescence.[12] These factors, together with inactive lifestyle, muscle loss and changes in bodily function, contribute to increased risk of clinically-significant infection.[13]

In many cases, an infection may be obvious by a physical examination, such as high fever or altered mental state[14] but some patients may show minimal clinical symptoms. For example, if a patient is already immunosuppressed, then they may not develop a fever despite an infection being present.[15] Certain medications, such as beta-blockers, can also mask symptoms and cause difficulty in diagnosing infection,[16] resulting in later-stage detection with corresponding higher morbidity and mortality.

The treatment of infection centres around source control and effective implementation of therapy (*e.g.* antibiotic, antiviral). The application of microorganisms for the treatment of microbial infections has been dated back to ancient Egypt, Greece and China, but the pivotal transition into modern era antibiotics was the discovery of penicillin by Sir Alexander Fleming in 1928.[17] Unfortunately, over-use of antibiotics has led to the promotion of antibiotic resistant bacteria. This was first reported in the early 1950s and now is reported for almost all antibiotics, causing infections to become more challenging to control and eradicate.[18] The World Health Organization has classed antibiotic resistance as a "global health threat" that is responsible for 700 000 deaths globally each year. Antibiotic resistance will always occur in new drugs, but the timelines for this is unpredictable.

1.2 Sepsis

In many cases, an infection will not be life threatening and can be effectively eradicated by a course of appropriate treatment.

However, in some cases it can lead to a cascade of pro- and anti-inflammatory responses that may cause organ dysfunction, permanent damage or even death if not controlled and treated promptly. Sepsis is then defined as "a life-threatening organ dysfunction due to a dysregulated host response to an infection".[19]

Sepsis may follow infection for example due to injury, burn or surgery. Seventy percent of cases result from bacterial origins compared to 20% from viral and 10% from fungal infection.[20] However, not all cases of an infection will result in sepsis. The determination of the cause of sepsis (*i.e.* bacterial, viral, or fungal) is useful to ensure appropriate and effective treatment. The National Confidential Enquiry into Patient Outcome and Death (NCEPOD) found that approximately 80% of sepsis cases in the UK were due to community-acquired infection.[21] At the onset of sepsis, pro-inflammatory pathways are dominant, with anti-inflammatory responses at later stages. Patient with sepsis will typically become immunosuppressed early, due to malfunctioning innate or adaptive immune systems.[22] The pathogenesis of sepsis at the molecular, cellular and organ levels has been reviewed in detail.[20]

Sepsis, severe sepsis and septic shock represent increasingly severe systemic inflammatory responses to an infection.[20] Septic shock is the most likely to be fatal as a result of extensive circulatory and metabolic abnormalities.[19] There has been debate over the connection of Systemic Inflammatory Response Syndrome (SIRS), sepsis and septic shock as a continuum of increasing severity, rather than classing them as individual conditions, since not all SIRS patients will subsequently develop septic shock.[23] Sepsis and SIRS can both lead to organ failure but by different physiological processes.

It is well documented that early and accurate identification of sepsis is crucial to successful treatment and outcome.[24–26] However, there is currently no gold standard diagnostic process for septic patients, so that identification relies on clinical suspicion together with physiological and laboratory tests. When sepsis is suspected, initial screening measurements of temperature, heart rate and respiratory rate are performed, followed by blood glucose, urine output, blood pressure, oxygen saturations and serum lactate measurements. Pathogenic blood cultures are also performed, followed by further investigations as required, such as imaging, urinalysis, or urine culture. Definite diagnosis of sepsis requires confirming the presence of an underlying infection through positive blood culture. Diagnosis of sepsis is made more challenging by overlap of clinical symptoms with other non-infectious conditions that share a systemic inflammatory response, such as pancreatitis, tissue ischemia, trauma and drug reactions.[20] Sepsis is, therefore, commonly under- or over-diagnosed and delayed initiation of treatment can have devastating impact, both on survival and on post-sepsis complications that occur in some 40% of cases.[21]

Low-income countries bear the greatest burden with cases of sepsis, primarily due to deprived living conditions with poor preventative healthcare, insufficient sanitation, malnutrition and a higher incidence of tropical and viral diseases.[27] These countries represent around 74% of global deaths relating to sepsis per year.[28] Factors such as lack of awareness of sepsis, limited access to treatment[29] and concomitant healthcare, and the prevalence of counterfeit medications[30] all contribute to poor patient outcomes. On an annual basis, there are approximately 30 million cases of sepsis reported globally, increasing by ~9% per year.[31,32] Over 6 million cases will result in death, although the real number is expected to be much higher, because of lack of information from low-to-middle income countries and inconsistencies in listing sepsis as the cause of death.[33]

In the UK, there are approximately 200 000 cases of sepsis annually, resulting in over 46 000 deaths,[21] representing 5–7% of all deaths.[34] The incidence is increasing by 11.5% per year,[21] and it is 6 times more expensive to treat septic than non-septic patients,[33] so sepsis represents a growing financial burden in both direct and indirect costs, the former being estimated as £2B per year.[21] Implementation of more effective diagnostic tools allowing sepsis to be identified within one hour of patient presentation is estimated to save the NHS upwards of £170M per year in care and treatment.[3]

1.3 Current diagnostic pathway

As discussed above, early identification of sepsis is critical to successful outcome. The management of sepsis relies on the early identification of the condition, with prompt control and eradication of the infection. The typical diagnostic pathway for infection and sepsis is illustrated in Fig. 1.

Microbiology testing is central to the diagnosis of infection and sepsis, enabling optimised targeted therapy. Since sepsis always arises from an infection, the only way to positively diagnose sepsis is by identifying the pathogen using blood cultures. However, these have several limitations: high volume of blood required (20–30 mL),[35] time for pathogen detection (2–3 d) and identification (5–7 d),[36] and antibiotic susceptibility testing.[37] Furthermore, only 6–15% of cultures from patients with a suspected infection will yield a positive result,[38] due to low circulating microorganisms and the intermittent nature of blood stream infections.[39,40] False positive results,[40] culture contamination[41] and low blood culture volumes[42] are also problematic. Hence, blood cultures are only taken in about 50% of cases of suspected sepsis.[38]

Efforts are being made to develop rapid diagnostic tools particularly for pathogen identification and determination of antibiotic resistance to allow for a more targeted approach. The polymerase chain reaction (PCR) technique has been the most prominent development in the quest for rapid pathogen capture and detection systems, by amplifying the pathogen signal. They are typically less time consuming than blood culture/colony counting, typically taking 5–24 h, depending on the specific method used.[43] Optical biosensors,[44] bioluminescent sensors,[45] and flow cytometry[46] have all been investigated for pathogen detection but pathogen amplification is still required in almost all cases. These techniques will be discussed in more detail below.

The use of SIRS criteria for diagnosing sepsis is not fully endorsed and it has been well documented that these criteria are not specific to an infectious cause, with up to 90% of acutely

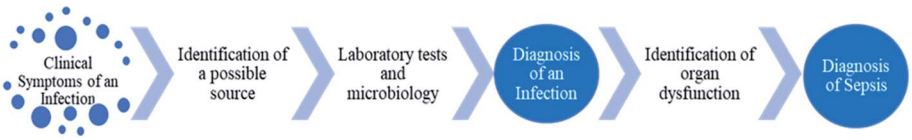

Fig. 1 Typical clinical pathway to the diagnosis of infection or sepsis. Adapted from ref. 15.

ill patients fulfilling the criteria even in the absence of an infection, for example following recent trauma or surgery.[47,48] This can lead to clinicians treating SIRS-positive and septic patients equally. However, the criteria are still relevant in initial patient assessment and identifying if the patient is likely to have an underlying infection and have laid the groundwork for more comprehensive sepsis assessment tools to be used for risk stratification in the initial assessment period. Details of these screening tools and their respective advantages and disadvantages are summarized in Table 2.

Patients identified as at risk for sepsis are then assessed to identify a possible infection if this is suspected. Pneumonia is the cause of some 50% of cases of sepsis, followed by urinary tract infections (20%) and abdominal (15%) infections.[21] To diagnose sepsis, one or more dysfunctional organs must be identified. The SOFA scoring system is currently the most appropriate method for identifying and quantifying organ dysfunction but requires two sequential scores, the first serving as baseline, around 24 hours apart.[49] Calculation of the SOFA score is a relatively time-consuming process, which is unrealistic in busy wards or emergency departments.[21] In this case, red-flag sepsis criteria are used to suggest the degree of organ dysfunction (*i.e.* high heart rate, low blood pressure and lactatemia). However, this is not a formal diagnostic method and the presence of two or more red flags can only suggest that the patient is very likely to have sepsis. Comprehensive guidelines to diagnose and treat sepsis have been published.[21]

Over 170 biomarkers have been studied for their potential to identify and diagnose infections and sepsis at earlier stages.[50] However, no biomarker exists that is specific only to sepsis. Typically, they are indicators of inflammation. As sepsis is a very complex condition with many inflammatory mediators and pathophysiological mechanisms, the time at which a specific biomarker would be diagnostically useful varies, which is a challenge in assessing biomarkers for their diagnostic and prognostic utility. The general census is that current biomarker quantification is more useful in ruling out rather than confirming infection. Nevertheless, the development of a multi-panel approach for biomarker quantification is appealing, as a more thorough diagnostic tool with potential applications in antibiotic stewardship. Table 3 illustrates key sepsis biomarkers investigated for diagnostic and prognostic capabilities, together with their advantages and limitations.

Time is a critical factor in the diagnosis and management of sepsis, with every hour of treatment delay increasing the risk of death by about 8%.[26] Despite ongoing improvements in patient management, there is high variability in the treatment of sepsis. There is a general agreement that treatment should be started as soon as possible after sepsis is suspected[24,51] but in most cases it is necessary to initiate treatment before the pathogen is identified. Thus, in bacterial sepsis, the patient is initially treated with broad-spectrum antibiotics before the results from blood cultures are available to optimise the choice and duration of the antibiotic therapy. Broad-spectrum antibiotics are not always sufficient, due to increasing antimicrobial resistance of many pathogens. However, the use of targeted, narrow-spectrum antibiotics is only made possible by rapid identification and/or classification of the pathogen.

Treatment of sepsis typically includes fluid management, antimicrobial therapy, respiratory support, and vasoactive drugs. The so-called sepsis 6 protocol to control infection and restore normal function, including oxygen delivery, is recommended within 1 h of suspicion of sepsis and reduces the overall mortality by 46.6%.[25] However, a review in 2015 found that only 63% of septic patients received antimicrobials therapy in the first hour following diagnosis and the delay led to negative outcomes for 44% of these patients.[52]

There is currently no well-defined protocol for pre-hospital emergency care of sepsis of sepsis which averages 45 min, whereby patients are transported to hospital by emergency services.[53] Hence, there is an unmet need for a portable, point-of-care device to help inform emergency personnel. In the UK, approximately 50% of patients who present to hospital emergency departments with sepsis arrived by ambulance[53] and typically have more advanced sepsis and higher risk of death. Screening tools and point-of-care tests are needed to stratify patients and facilitate delivery of effective care.

Current diagnostic procedures for sepsis are insufficient and cause delays in diagnosis and treatment of critically-ill patients.[54,55] New diagnostic devices are needed that fit with healthcare workflows by providing a means for earlier diagnosis.

2. Vibrational spectroscopy for infection and sepsis detection

Vibrational spectroscopy comprises the complementary techniques of infrared (IR) and Raman spectroscopy, using light to probe intramolecular vibrations and rotations of a sample such as a biospecimen. The vibrational frequencies of specific molecules are unique, allowing molecular "fingerprinting" of the biomolecular composition of the sample allowing a means to deduce its chemical and structural components. This represents an opportunity for non-invasive medical diagnostics. Such measurements, supported by computational analysis, should allow point-of-care devices to be used by clinical personnel with minimal training and expertise.

Table 2 Current protocols and diagnostic criteria used to diagnose infections and sepsis

Diagnosis protocol	Criteria	Advantages	Limitations
SIRS criteria	• Temperature	✓ Still relevant for identifying an infectious source	✗ Poor specificity – 90% of ICU patients and 50% of general ward patients fulfil criteria[48,109]
	• Pulse rate • Respiratory rate • White blood cell count • Blood glucose • New confusion	✓ Can be used to define "uncomplicated sepsis" which is without evidence of organ dysfunction[21]	✗ Sensitivity of 81% and specificity of 41% for predicting mortality in ED patients with infections[110]
Sepsis-3	• Persisting hypotension	✓ Accurate at identifying very high-risk. Patients who have a higher mortality risk	✗ Requires the presence of organ dysfunction to diagnose sepsis
	• Elevated serum lactate • Organ dysfunction identified by SOFA	✓ Identifies organ failure	✗ Fails to identify patients with serious infections before organ dysfunction is established[111]
Sequential Organ Failure Assessment (SOFA)	Organ systems analysed:	✓ Accurately identifies organ dysfunction	✗ Requires two scores minimum – on admission and at each 24 hour period that follows
	• Respiratory	✓ Assesses each of the 6 organ systems to identify organ dysfunction	✗ Very time consuming
	• Cardiovascular	✓ Increase in SOFA score by 2 or more is indicator of in hospital mortality. Sensitivity 76%, specificity 70% (ref. 112)	✗ Fails to identify sepsis at early stages
	• Renal • Neurological • Hepatic • Haematological		✗ Not used outside of the ICU ✗ Requires laboratory values which may not be readily available
Quick-SOFA (q-SOFA)	• Respiratory rate	✓ Used to prompt clinicians to "think sepsis"	✗ Typically identifies high risk patients too late
	• Systolic blood pressure • Altered mental state	✓ Simple and quick ✓ Specificity of 96% for predicting in-hospital mortality on admission into ED[113]	✗ Sensitivity of 21% for predicting in-hospital mortality on admission into ED[113]
National Early Warning Score (NEWS)	• Respiratory rate	✓ Useful in ruling out sepsis	✗ Low positive predictive value (35.4%) for detecting clinical deterioration[115]
	• Oxygen saturation • Body temperature • Systolic blood pressure • Heart rate • Supplemental oxygen • AVPU score	✓ Easy to use without increasing workload ✓ Does not require blood test results ✓ Accurate of 77% for predicting in-house mortality[114]	✗ Brain function depends solely on AVPU and does not discriminate between degrees of responsiveness (sleepiness and extreme unresponsiveness are treated identically)[116]
Simplified-MISSED	• Age ≥65 years	✓ Very simple and easy to calculate	✗ Relatively new system that requires further multi-centre validation
	• Serum albumin • International normalised ratio	✓ Complements already established sepsis criteria ✓ Independent of comorbidities in predicting mortality[117]	✗ Serum albumin and INR can be affected by the impaired synthetic function of the liver[118]
Risk-stratification of emergency department suspected sepsis (REDS) score	• Age ≥65 years	✓ Easy to calculate in a busy ED	✗ Sensitivity of 38% for predicting in-hospital mortality on arrival to ED[113]
	• Altered mental state	✓ Embraces current definition of septic shock	✗ Can only be calculated once sepsis-6 have been completed and blood test results are available[119]
	• Systolic blood pressure • Respiratory rate • Lactate • Albumin • International normalised ratio • Refractory hypotension	✓ Specificity of 89% for predicting in-hospital mortality on admission into ED[113]	✗ Requires further multicentre validation

Table 3 Key biomarkers assessed for diagnosing infections and sepsis

Biomarker	Suitability	Advantages	Limitations	Current applications
C-Reactive Protein (CRP)	Synthesised in the liver in response to stimuli by cytokines, mainly including IL-6	✓ Quantification algorithms can shorten antibiotic treatment duration by ~1.45 days (ref. 120)	✗ Low specificity (~33.3%) for infection[123]	Measured using assay systems to illustrate inflammation. 5 mL of blood taken in gold top tube
		✓ Useful in predicting mortality risk[121]	✗ Elevated levels in burns, trauma, surgery and non-infectious conditions	
		✓ CRP levels typically double every 8 hours after endotoxin challenge[122]	✗ Marker of inflammation rather than infection	
Lactate	Reflects the balance between lactate production and clearance	✓ Levels >4 mmol L^{-1} indicate a relative risk of mortality of 40% (ref. 124)	✗ Not specific to sepsis	Normally assayed in blood gas analyser machines. 2 mL of blood is taken in a fluoride-oxalate tube
		✓ Useful in quantifying the level of organ failure	✗ Lactate levels can be high due to malignancy, liver disease, mitochondrial disorder[126]	
		✓ Sensitivity of 82.9% and specificity of 83.4% for predicting sepsis[125]		
Procalcitonin (PCT)	Produced in the thyroid cells and then converted to calcitonin. Very low levels in healthy patients. Released within 3–4 hours after inflammatory stimulus[123]	✓ Bacterial endotoxins are a key stimulus of the synthesis of PCT and can be used as an indicator for bacterial infections[122]	✗ No set cut-off value for defining sepsis	Measured using an immunofluorescent assay. Not routinely available in NHS currently. 3.5 mL of blood taken in rust top gel tube
		✓ Sensitivity of 72.7% and specificity of 81.7% for predicting sepsis[67]	✗ Assays are expensive and are prone to false results	
		✓ Demonstrated to reduce the duration of antibiotics by ~2.7 days[127]	✗ Variation in PCT assays and predictive cut-off points cause difficulties in interpretation of results[128]	
Neutrophil (count)	Constitute 50–70% of all circulating white blood cells. They are the first cells to respond to an infection	✓ Already used in the definition of sepsis and diagnostic criteria	✗ 62.9% sensitivity and 62.8% specificity for predicting sepsis[125]	Differential white blood cell count provided by automated haematology machines in hospital laboratory. Up to 5 mL of blood collected in lavender top tube (EDTA)
		✓ Physiological immune response characterised by an increase in neutrophil and white blood cell count	✗ Neutrophil count may be very high or very low if the patient has an infection	
Immature Granulocyte (IG) (count)	Number of white blood cells with granules in their cytoplasm and two or more lobes in their nuclei. Inclusive term for neutrophils, basophils, and eosinophils	✓ Provides information on bone marrow activity	✗ Sensitivity of 60% and specificity of 67% for predicting sepsis[125]	Automated haematology machines in hospital laboratory or manual microscopy counts can be used to calculate IG count. Up to 5 mL of blood collected in lavender top tube (EDTA)
		✓ Healthy individuals do not have IG present in peripheral blood	✗ Low predictive values for prognosis and mortality	
		✓ Discriminates between infection and non-infected patients with a sensitivity of 89.2% and specificity of 76.4% (ref. 129)		
Calprotectin	Released upon neutrophil activation. Constitutes between 40–60% of the soluble cytosolic protein found in neutrophils[130]	✓ Increased concentration within hours after bacterial or endotoxin challenge[131]	✗ Specificity of 56% for discriminating between sepsis and non-sepsis[131]	Serum calprotectin is not currently routinely measured in the NHS. Faecal calprotectin is used to distinguish between inflammatory bowel disease and irritable bowel syndrome
		✓ Sensitivity of 81% for discriminating between sepsis and non-sepsis[131]	✗ Low specificity (46%) for predicting 30 day mortality[131]	
		✓ High predictor of 30 day mortality (80% sensitivity)[131]		

In the diagnosis of infections, it is critical to identify the specific microbe species (bacterial, fungal, or viral) to inform the choice of treatment. Emerging technologies for this application include the use of PCR (polymerase chain reaction to amplify the measurement signal by replicating the genetic material in the sample), flow cytometry (detects and measures

physical and chemical characteristics of cells), optical biosensors (detection of optical signals that are proportionate to concentration of analyte under investigation) and bioluminescent sensors (detection of light produced following chemical reaction with biological material). Flow cytometry and biosensor techniques typically involve a pathogen amplification step prior to analysis. A detailed critical review of these techniques for pathogen detection has been published by Rajapaksha *et al.*[43] Assay techniques (such as molecular based assays or PCR-assays) have been the most studied for rapid identification of microbes, with high specificity (95%) but are labour intensive, slow (up to 16 h) and expensive[43,56] and also require trained technical personnel.[57] Flow cytometry is quantitative and yield high sensitivity in <1 h.[43] Its limitations are the need for complex sample preparation, high equipment costs and the need for expert operators.[43,46] Optical biosensors and bioluminescent sensors allow rapid, high-throughput pathogen detection and quantification of but have poor detection limits and low sensitivity.[44] Mass spectrometry has also been investigated for rapid (<1 h) microbial identification, with low detection limits (~16 colony forming units (CFU) per mL).[58–60] However, it has limited ability to differentiate bacterial species[61] and requires extensive sample preparation compared to other techniques.[62]

In principle, vibrational spectroscopies can overcome various of these limitations, by providing a label-free analytical method with simple sample preparation, low sample volumes (typically ~μL) and the ability to obtain a molecular fingerprint of the full sample. A comparison of the analytical steps required for the current gold-standard technique of blood culture, the above emerging assay techniques, and the proposed vibrational spectroscopic techniques is presented in Table 4. Although Table 4 shows the case for bacteria only, all techniques are applicable to other pathogens including viruses, yeast, and fungi.[63–65] It should be noted that drying of the sample is required for IR spectroscopy[66] but is not necessary in Raman spectroscopy. However, some reduction of the biofluid water content increases the microbe concentration and, hence, the signal strength.[67]

Significant early research in the application of vibrational spectroscopy to infection diagnosis was carried out by Naumann and colleagues, who demonstrated such techniques to be powerful analytical tools for the identification and characterisation of microbial cells by the determination of spectral characteristics from the microbes and the characteristic absorptions of the cellular proteins of the bacteria.[68,69] However, this use of Fourier Transform Infrared (FTIR) spectroscopy for whole-cell bacterial samples is confounded by the effect on the acquired spectra of the culture conditions (medium, temperature and time).[70]

Much of the work on identifying pathogenic bacterial strains has been accomplished by Raman spectroscopy,[71,72] including surface-enhanced Raman spectroscopy (SERS) using gold or silver nanoparticles to amplify the otherwise weak Raman signal. Numerous studies have shown the ability SERS to identify and differentiate invading pathogens[73] and quantify live and dead bacteria populations.[74] FTIR microspectroscopy has demonstrated to detect and identify bacterial and yeast microcolonies[75] with classification accuracies upwards of 80%.[76] More recently, FTIR spectroscopy has been reported to identify and classify different microbial strains[77] and also identify Gram-negative bacterial species responsible for hospital outbreaks within 3 h, using second derivative amplification clustering methods whereby microbials are classified into distinctive groups following amplification of the differences between isolates.[78] Additionally, FTIR has shown utility in distinguishing between bacteria, fungus and viruses microorganisms.[64,79]

In the diagnosis of sepsis, both the invading pathogens and host-response biomarker concentrations are diagnostically important to determine treatment options. Potential biomarkers are listed in Table 3. The identification of sepsis using vibrational spectroscopy can be achieved by detecting the optical signatures of both the pathogens and the host biomarkers. To date, a very limited number of studies have considered the application of vibrational spectroscopic techniques to diagnose sepsis. Raman spectroscopy has been used to distinguish between non-infectious SIRS and sepsis using

Table 4 Comparison of techniques for detection of pathogenic bacteria in clinical analysis

Analysis step	Blood cultures	Assay techniques	IR spectroscopy	Raman spectroscopy
Preparation time	<15 minutes (ref. 35)	<1 hour (ref. 40)	15–25 minutes (ref. 77 and 78)	<20 minutes (ref. 72)
Volume of blood	20–30 mL per set (ref. 132)	<5 mL (ref. 40)	1–5 μL (ref. 77)	1–10 μL (ref. 72 and 133)
Culture time	2–7 days (ref. 35, 40 and 134)	24 hours (ref. 45)	6–24 hours (ref. 77 and 135)	6–24 hours (ref. 72, 73 and 133)
Drying time	N/A	N/A	<30 minutes (ref. 78 and 135)	0–60 minutes (ref. 73 and 133)
Analysis time[a]	<2 hours[b] (ref. 35)	4–16 hours (ref. 40)	<20 minutes (ref. 136 and 137)	<30 minutes (ref. 138 and 139)
Positive predictive value	<55% for 1 culture, <98% for 2 (ref. 140)	30–57% (ref. 56, 141 and 142)	<97% (ref. 136)	<97% (ref. 71)

[a] Analysis time following blood culture. [b] Analysis time for identification of microorganisms by gram staining following alert of positive blood culture result. Additional time (24–48 h) is required to determine antibiotic susceptibility.

blood plasma samples, with a prediction accuracy of 80%.[80] FTIR micro-spectroscopy has allowed the analysis of sepsis progression in mouse models.[81] Spectral changes in saliva were observed between control and septic neonatal patients using FTIR.[82] However, vibrational spectroscopic techniques are still currently limited by the requirement of culturing due to the low concentrations of circulating microbes within biofluids.

It has also been shown that detection of biomarkers can aid in the identification of infections and sepsis before clinical symptoms present.[83] This would be of particular benefit in the intensive-care setting (ICUs), where patients are at a high-risk of developing infections and resulting sepsis. FTIR spectroscopy has been investigated to quantify glucose and lactate in whole blood and blood serum samples.[84,85] Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy has been shown to be useful in quantifying protein concentrations within blood serum samples.[86] There is an extensive literature on the spectral analysis of biomarkers to aid in the diagnosis of various types of cancer, but there a lack of studies on infection biomarkers, such as C-Reactive Protein (CRP) and procalcitonin (PCT), that would be useful in diagnosing infections and sepsis.[50]

A primary requirement for vibrational spectroscopy-based devices is that they generate rapid, clinically actionable results with high sensitivity and specificity. This requires detection across a wide range of pathogen concentration, say 1–100 000 CFU mL^{-1}.[40] Appropriate device design is crucial for successful translation of new medical devices. In a generic sense, visualisation of results, necessary level of training, expertise required, and maintenance requirements must be appropriate for the end user. Furthermore, the device must be suitable for its environment of use with regards to its accessibility, physical footprint and the necessary continual costs for device use and upkeep. Translation of vibrational spectroscopic techniques to the routine clinical environment remains elusive, despite the large number of studies demonstrating the performance and capabilities of such techniques for other clinical applications.[87,88] However, significant modification of these technologies are required for high-throughput, streamlined and user-friendly use in the infection/sepsis setting[89] and the methods and devices will require extensive multicentre trials validate the measurements and analyses. The general challenges in clinical translation of infrared and Raman spectroscopy have been reviewed elsewhere.[66,89]

As mentioned above, vibrational spectroscopy techniques are currently still limited by the requirement of culturing before spectral acquisition and analysis, due to the typically very low concentrations of microbes within liquid bio-samples such as blood (1–10 CFU mL^{-1}).[39] Efforts are being made to eliminate or reduce this requirement for the clinical environment.[72]

Grey areas remain in the diagnosis of sepsis, as opposed to diagnosis of infection: in the definite presence or absence of the condition[15] and a complete diagnostic platform will require integration of multiple diagnostic criteria, along with clinician expertise. Vibrational spectroscopy has the potential to contribute significantly to the overall diagnostic process by providing high-throughput, label-free, non-destructive and low cost detection of pathogenic microorganisms and endogenous biomarkers of sepsis, impacting the 14 000 annual deaths in the UK due to sepsis could have been prevented by earlier diagnosis and intervention.[90]

3. Proposed development pathway

Health care systems, including in the UK, are under considerable strain, with ever increasing resource demands to treat more patients with the highest quality of care while minimizing costs. On average, a patient is checked every 4 h for signs of sepsis and infection in ICUs in UK hospitals, during which time a patient's condition can deteriorate to become life-threatening.[91] Rapid testing has potential to inform clinical care, including escalation or change of therapy. It is evident that accurate and reliable analysis of small blood samples at point-of-care would represent a pivotal development. The technology would also be useful in creating a baseline at time of presentation to monitor progress or deterioration, especially it if can fit seamlessly into the clinical workflow.

We propose specifically for the diagnosis of sepsis the development of a point-of-care device that is capable of rapid blood analysis using vibrational spectroscopy by providing critical data on differential white blood cell counts and key infection biomarkers. This approach for the early identification of sepsis would complement clinical judgment to provide a more thorough diagnostic approach. There is also scope for this technology to be adapted for spectral identification of microbes, and for detection of antimicrobial resistance to guiding antibiotic therapy. At present, UK metropolitan hospitals aim to provide full blood counts from patients in emergency within a minimum time of 40 min from receipt of the sample in the lab but in reality this often takes over an hour.[92] A novel point-of-care diagnostic tool must be then capable of producing a result in much less time, which is a significant technical challenge. However, if the technology barriers can be overcome, then there are multiple additional clinical applications. One example is routine blood analysis within healthcare centres and family practices. Since an appointment with a family doctor lasts typically about 10 min, this represents a target turn-around time for the diagnostic test, avoiding the need to send samples to a diagnostic laboratory, thereby easing the burden on hospital labs that could then give priority to the most critical-ill patients. There is also scope for such a device to be used in remote and rural clinics, field hospitals and in low-resource settings. Additionally, its use by paramedical and first-responder personnel could allow patients to be flagged before reaching the emergency department, so as to allow immediate treatment and stabilisation.

4. Conclusions

The application of vibrational spectroscopy in the quest of early disease diagnostics has been a major focus in recent years, due to its rapid and non-invasive nature and cost-effectiveness. Although currently not fully translated into the clinical environment, there is significance for early diagnostics, patient

monitoring, and treatment guidance. This review has highlighted the potential for point-of-care spectroscopic devices for blood the analysis in particular, to aid in the diagnosis of infections and sepsis by providing a rapid and accurate tool to quantify sepsis-related biomarkers and to detect/identify the causative pathogens. The current diagnostic pathway involves numerous steps for identification of infections and sepsis, and uses technologies requiring extensive sample preparation and analysis time. It has been well recognized that these current methods are inadequate and cause significant delays in diagnosis and initiation of effective treatment, that negatively impacts the staggering number of hospitalisations and deaths worldwide each year (30 and 9 million, respectively[32]). Earlier diagnosis of sepsis would save more than 14 000 lives annually and reduce healthcare costs by upwards of £170 million in the UK alone.[3,90] Vibrational spectroscopy can, in principle and likely also in practice, overcome these limitations by providing a rapid, label-free analytical tool for point-of-care blood analysis, and is the focus of ongoing research and development.

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Conflicts of interest

There are no conflicts of interest to declare.

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