

THE INTERACTION OF NEAR-INFRARED LIGHT WITH BIOLOGICAL SAMPLES TO GAIN VALUABLE INSIGHTS INTO MOLECULAR COMPOSITION AND STRUCTURE

Muhammad Yousaf^{1*}, Ghulam Murtaza²

¹Chemistry Department, COMSATS University Islamabad, Lahore Campus, Pakistan

²Department of Chemistry, University of Bath, UK.

Submitted November 8, 2024; Revised December 21, 2024; Accepted December 22, 2024; Published January 20, 2025

ABSTRACT

Near-infrared (NIR) spectroscopy is emerging as a rapid, non-invasive diagnostic tool for infectious diseases in both humans and animals. This technique analyzes the interaction of NIR light with biological samples, providing valuable insights into molecular composition and structure. Recent advancements, particularly the integration of chemometrics and artificial intelligence, have greatly improved the interpretation of spectral data and the accuracy of diagnoses. The development of portable NIR devices further facilitates its application in resource-limited environments. In comparison to traditional diagnostic methods, NIR offers quicker results and the capability to assess multiple biological components simultaneously. However, challenges such as limited sensitivity to trace biomarkers, sample complexity, instrument calibration, environmental factors, and the absence of standardized protocols persist. Despite these challenges, NIR spectroscopy has significant potential to enhance the diagnosis and monitoring of infectious diseases. This review explores the principles of NIR spectroscopy, its current applications, advantages, technological advancements, limitations, and its future as a practical, low-preparation diagnostic method.

Keywords: Near-infrared spectroscopy, infectious diseases, diagnosis, monitoring

*Corresponding Author. E-mail: sonofchaudary.2003@gmail.com

INTRODUCTION

According to the latest reports from the World Health Organization, infectious diseases remain a major contributor to global morbidity and mortality. In 2021, COVID-19 alone resulted in 8.8 million deaths, making it the second leading cause of death worldwide. As a result, the proportion of deaths attributed to infectious diseases rose to 28.1%, up from 18.2% in 2019. Lower respiratory infections

also posed a significant threat, ranking as the fifth leading cause of death with 2.5 million lives lost in 2021. In low-income countries, diseases like malaria, tuberculosis, and human immunodeficiency virus (HIV) continued to drive high mortality rates, with HIV alone affecting 39 million people globally by the end of 2022 [1]. Additionally, infectious diseases in animals present substantial risks, impacting

PST is published by AMMANIF Publisher. The authors retain the copyright without restriction. This article is an open access article published under the terms and conditions of [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).



livestock health and agriculture, and potentially leading to zoonotic transmissions that can endanger human populations. Zoonotic diseases affect over two billion people worldwide and result in more than two million deaths each year. The economic impact of infectious diseases is staggering; major outbreaks like severe acute respiratory syndrome, swine influenza, and Ebola have historically cost billions in healthcare, lost productivity, and economic disruptions. For instance, the COVID-19 pandemic caused a global economic contraction of 4.3% in 2020, resulting in a loss of \$3.6 trillion in goods and services. This underscores the urgent need for rapid diagnostics and accurate identification of infectious diseases in both humans and animals to control outbreaks and guide effective treatment strategies, ultimately reducing morbidity, mortality, and healthcare costs [2].

Recognizing the critical role of early and accurate diagnosis in mitigating the impact of infectious diseases, numerous diagnostic methods have been developed and refined over time. Molecular techniques such as polymerase chain reaction, DNA probing, and plasmid profile analysis were introduced decades ago to address the limitations of traditional culture techniques. These methods are highly sensitive and specific, allowing for the diagnosis of infectious diseases at the genetic level; however, they require extensive sample preparation, sophisticated laboratory facilities, and skilled personnel. In contrast, spectroscopic methods offer several advantages for diagnosing infectious diseases by providing faster, real-time results without the need for complex multi-stage sample processing. These techniques are versatile, capable of analyzing a wide range of biochemical constituents within a single test. For example, infrared (IR) and Raman spectroscopy can quickly analyze blood, tissue, or other biological samples without extensive chemical preparation. However, the

application of IR spectroscopy in biological systems is often limited due to strong water absorption in the IR region [3]. IR and Raman spectroscopy together provide complementary molecular information, making these approaches particularly suitable for point-of-care diagnostics. Moreover, these spectroscopic techniques are not restricted to detecting a single type of biomolecule; they can simultaneously analyze a diverse array of biochemical constituents, including proteins, lipids, and nucleic acids. This multi-component analysis is crucial for identifying complex infection profiles and monitoring disease progression.

NIR spectroscopy occupies a distinctive role among spectroscopic methods for the rapid diagnosis of infectious diseases, thanks to its unique spectral characteristics and processing capabilities that set it apart from other techniques like IR and Raman spectroscopy. This powerful vibrational technique utilizes the near-infrared region of the electromagnetic spectrum (800–2500 nm) to extract molecular information from biological samples by analyzing their characteristic spectral patterns, which arise from the absorption of overtones and combinations of stretching-bending vibrations of biomolecules. Within the NIR region exists an “optical window,” or “NIR window,” with wavelengths from 650 to 1100 nm. This third overtone region features relatively low tissue scattering and absorption, allowing light to penetrate deeply into tissues, making NIR spectroscopy ideal for noninvasive measurements of biological systems [4]. Consequently, NIR spectroscopy has the potential to rapidly and noninvasively diagnose infectious diseases—such as bacterial, viral, and parasitic infections—by analyzing molecular changes and compositions in biological samples like blood, tissue, and other body fluids, or by directly examining pathogens.

Recent advancements in NIR spectroscopy have significantly improved its application in

diagnosing infectious diseases. The development of miniaturized spectrophotometers has made NIR technology more portable and accessible, facilitating point-of-care testing in resource-limited settings and enhancing its utility for rapid diagnostics. These compact devices maintain high sensitivity and accuracy while offering convenience and ease of use. Moreover, advancements in spectral analysis methods, particularly through the integration of artificial intelligence, have refined data interpretation. Recent studies have shown that these advancements enable NIR spectroscopy to deliver detailed molecular information about infectious diseases, such as pathogen identification and disease progression, which are often difficult to obtain using traditional molecular methods [5-8].

This review aims to provide a comprehensive overview of NIR spectroscopy in the rapid diagnosis of infectious diseases in both humans and animals, including SARS-CoV-2, HIV, paratuberculosis, and bovine respiratory disease. It highlights the principles, current applications, advantages, recent advances, limitations, and future prospects of NIR spectroscopy as a promising method for low sample preparation and rapid disease diagnostics, emphasizing the integration of computer science and artificial intelligence in advancing this technology.

PRINCIPLES OF NIR SPECTROSCOPY FOR INFECTION DIAGNOSIS

The analysis of biological samples using NIR spectroscopy focuses on the study of molecular vibrations, which occur at specific frequencies determined by the masses of atoms, the strength of chemical bonds, and the molecular environment. Each functional group in biomolecules—such as hydroxyls, alkanes, and amines—vibrates at distinct characteristic frequencies.

A related technique, infrared absorption spectroscopy (IR), measures the absorption of infrared light at frequencies that correspond to the energy needed for molecules to transition between vibrational states. These transitions follow selection rules based on changes in the dipole moment, resulting in specific absorption peaks. The intensity of these peaks depends on the extent of dipole moment changes during vibration and the level of anharmonicity present. In the NIR region, higher energy overtones (absorptions at multiples of the fundamental frequency) and combination bands (interactions between different vibrational modes) are accessible, offering insights into molecular vibrations that are not easily observed with mid-infrared spectroscopy. NIR spectral features primarily arise from these overtones and combination bands, which are generally weaker than fundamental vibrations. The intensity of first overtones can be reduced by factors ranging from 10-100, while higher overtones and combination bands tend to be even less intense. However, bonds involving hydrogen atoms and heavier elements, such as C-H, O-H, S-H, and N-H, demonstrate considerable anharmonicity and strong bond energies. Their fundamental vibrations occur around 3000–4000 nm in the mid-infrared region, resulting in relatively strong absorptions in the NIR region from the overtones and combination bands of these bonds. This enables the detection of non-hydrogen-bonded species in dense solutions or liquids, as free overtones and combination bands produce distinct signals. Additionally, this capability is crucial for studying hydrogen bonding. By analyzing the absorption patterns of these overtones and combinations, NIR spectroscopy can identify and quantify various functional groups and molecular components within a sample [9].

NIR light, which operates in the "optical window" region, penetrates deeply into tissues—typically several millimeters to a few centimeters—making it ideal for analyzing molecular changes in internal structures such as the brain, muscles, blood vessels, and skin. In contrast, UV and visible light are absorbed more at the surface, limiting their penetration into deeper tissues. Furthermore, NIR light is less absorbed by water and hemoglobin, which are prevalent in biological tissues, compared to other types of radiation. For instance, IR light is strongly absorbed by water, while visible light is mainly absorbed by hemoglobin. This allows NIR light to penetrate deeper into tissues more efficiently and provides clearer analysis of molecular vibrations in internal structures. Additionally, NIR light scatters less than UV or visible light when interacting with biological tissues, enabling more of the light to penetrate deeper without deflection. Consequently, reduced scattering enhances tissue penetration and clarity when examining internal structures, making NIR spectroscopy a valuable tool in diagnostic applications [10].

CHEMOMETRICS FOR ENHANCED NEAR-INFRARED SPECTRAL ANALYSIS

NIR spectral data analysis can be categorized into two primary approaches, direct analysis and indirect analysis. The direct analysis approach involves straightforward observation of specific NIR absorption spectra characteristic of the material being studied. For instance, a distinct absorption peak at 970 nm is commonly associated with water, while a peak at 930 nm indicates adipose fat content. Direct analysis is useful for quick assessments where specific spectral features are known to correlate with particular substances, allowing for immediate qualitative insights. In contrast, the indirect

analysis approach employs more sophisticated methods, utilizing chemometric techniques that apply mathematical and statistical methods to extract meaningful data from spectral variations by correlating them with the material's composition [11-13]. Chemometric methods, when integrated with artificial intelligence and machine learning, enhance the analysis and interpretation of complex spectral data. This approach effectively detects and quantifies substances, especially when direct observation is inadequate. By applying chemometric techniques, researchers can interpret more complex data sets to reveal detailed information about samples, categorizing these techniques based on their applications in data preprocessing, functions, and the types of analyses they perform.

Chemometrics is crucial in data preprocessing, offering systematic methods to refine and analyze spectral data. It employs techniques such as smoothing algorithms, normalization, and baseline correction to ensure that the data is clean and standardized. By removing noise, correcting for baseline shifts, and adjusting for varying scales, chemometric methods enhance data quality, ultimately improving the accuracy of subsequent analyses.

PORTABLE NIR SPECTROMETERS FOR INFECTIOUS DIAGNOSIS

Infectious diseases necessitate rapid and accurate diagnostic tools to prevent outbreaks and facilitate timely treatment. Miniaturized near-infrared (NIR) devices, comparable in size to smartphones, have transformed the field by enabling real-time, on-site analysis. These handheld devices can be employed at the point of care, allowing for quick assessment of biological samples for pathogens or disease biomarkers. Their portability and user-friendliness make them particularly suitable

for environments that require immediate diagnostic results. The miniaturization of benchtop NIR spectrometers into portable devices has been propelled by advancements in several components, including light sources, wavelength selectors, and detectors.

NIR spectroscopy systems generally consist of three key components: a light source, a wavelength selection unit, and a detector. The light source emits broad-spectrum light in the near-infrared region. The wavelength selector isolates specific spectral bands of interest, while the detector converts transmitted or reflected light into measurable signals that form the NIR spectrum [14].

Traditional benchtop NIR spectrometers use large, power-intensive light sources such as tungsten halogen bulbs, which provide high brightness and a wide spectral range. However, these bulbs generate significant heat, making them unsuitable for portable devices. To address this issue, smaller, energy-efficient light-emitting diodes (LEDs) have been developed, consuming less power and producing minimal heat. Although early LEDs had a narrower emission range, recent advances have resulted in NIR-specific LEDs that cover the necessary wavelengths for various diagnostic applications.

Benchtop NIR spectrometers typically employ wavelength selection techniques like diffraction gratings, prisms, and Fourier transform interferometers to disperse or modulate light across the desired spectral range. The miniaturization of NIR devices has been accelerated by innovative wavelength selection methods. For instance, Micro-Electromechanical Systems based Fabry-Perot interferometers use micro-machined, electronically controlled mirrors to filter specific wavelengths precisely, thus eliminating the need for large, moving components. Linear variable filters, which

vary the transmitted wavelengths along a small surface, enable compact and cost-effective wavelength selection without mechanical parts. Digital micromirror devices, consisting of an array of tiny mirrors that rapidly tilt to direct specific wavelengths, offer highly accurate and fast wavelength control in a compact design. Meanwhile, Hadamard transform spectrometers use encoded light patterns with mathematical decoding to facilitate multiplexed wavelength detection and enhance sensitivity, all without requiring large optical systems. Collectively, these technologies enable smaller, more portable NIR devices that maintain the performance of traditional benchtop spectrometers, making them ideal for real-time, on-site diagnostics and point-of-care applications [15].

Recent advancements in detector technology have significantly contributed to the miniaturization of near-infrared spectrometers. Traditionally, benchtop devices relied on larger, more sensitive detectors, such as extended Indium Gallium Arsenide detectors, which cover the mid to long-wave NIR range. While essential for capturing NIR signals, these detectors typically require cooling systems to maintain performance, resulting in heavy and cumbersome units. However, innovations in solid-state detector technologies, including smaller Indium Gallium Arsenide sensors and silicon-based detectors, have drastically reduced their size while enhancing performance. Additionally, advances in complementary metal-oxide-semiconductor technology have facilitated the development of compact, energy-efficient detectors that enable portable devices to maintain high sensitivity and precision without the need for extensive cooling systems.

Moreover, modern handheld NIR devices are increasingly user-friendly, featuring

functionalities that make them accessible to healthcare workers with minimal training. Many of these devices incorporate software solutions that streamline data analysis, offering real-time diagnostics through mobile applications [16]. They also leverage wireless communication technologies like Bluetooth and Wi-Fi, which allow data transmission to smartphones or cloud platforms for further analysis, thus eliminating the need for bulky computers and further accelerating the miniaturization process.

DIAGNOSTICS OF INFECTIOUS DISEASES WITH NIR SPECTROSCOPY

Viral Infections

Traditional diagnostic methods, including enzyme-linked immunosorbent assay, immunofluorescence assay, reverse transcription-polymerase chain reaction, and Western blotting, are widely utilized to identify viral infections. NIR spectroscopy complements these techniques by detecting changes in molecular composition caused by viral infections, which manifest as distinctive spectral signatures. During spectral analysis, optimal wavelengths are selected for their sensitivity to sample concentration changes, with notable absorption peaks for water (or O-H vibration) at 970, 1400, and 1900 nm relevant for viral detection.

NIR spectroscopy is emerging as an advanced technique for detecting viral infections by leveraging the unique spectral characteristics of viral components, including nucleocapsids and envelope proteins. This method distinguishes between infected and uninfected individuals by identifying specific absorption peaks that vary with sample concentration. NIR spectroscopy has demonstrated potential for accurate viral identification, highlighting its value in virology. It has been effectively

used to detect viruses such as SARS-CoV-2, HIV, influenza, and Zika [17].

A pilot study aims to diagnose COVID-19 using NIR spectroscopy. The process involves collecting samples from patients *via* throat or nasal swabs, saliva, or blood, with nasal samples often exhibiting higher viral loads. The NIR spectroscopic instrument collects spectral data, focusing on specific wavelengths sensitive to SARS-CoV-2. Chemometric models and machine learning techniques, such as PCA/PLS, are utilized to differentiate SARS-CoV-2 from other viruses exhibiting similar symptoms. The NIR spectrometer system is optimized by adjusting optical components like fiber diameters and lenses to enhance specificity and sensitivity for accurate pre-diagnosis of infections.

The study explored the use of optical spectroscopy in the visible and near-infrared range, combined with machine learning techniques, to identify SARS-CoV-2. Data preprocessing included Savitzky-Golay derivative analysis, Principal Component Analysis, and Partial Least Squares Regression, to refine signals and extract significant features. Machine learning models such as Random Forest, Support Vector Machine, and Naive Bayes classifiers were employed for sample classification. The effectiveness of these models was evaluated using 5-fold cross-validation, resulting in better sensitivity, accuracy, specificity and an ROC curve area. The findings suggest that Vis-NIR spectroscopy is a relatively inexpensive and rapid method for detecting SARS-CoV-2.

NIR spectroscopy has also been used to determine the age and diagnose mosquito-borne arboviruses, including Zika, dengue, and chikungunya. The first study exploring the noninvasive detection of Zika virus in *Aedes aegypti* mosquitoes was conducted. They fed mosquitoes with ZIKV-infected or

uninfected blood and collected NIR spectra from the head/thorax regions at 4 and 7 days post-infection. The virus's presence was confirmed with RT-qPCR, and NIR spectroscopy distinguished infected from uninfected mosquitoes with up to 99.3% accuracy. This method is faster and more cost-effective than RT-qPCR, allowing for rapid, high-throughput screening by unskilled technicians. The study highlights NIR spectroscopy's potential as an efficient tool for detecting viral infections in mosquitoes, aiding in disease outbreak control. Garcia et al. conducted another study that detected DENV and ZIKV infections in *A. aegypti* mosquitoes by analyzing infection-induced physiological changes through NIR spectroscopy, achieving high accuracy in differentiating between uninfected mosquitoes and those infected with DENV, ZIKV, or both [18].

One strategy to reduce the spread of arboviruses is to release *A. aegypti* mosquitoes infected with the bacterium *Wolbachia pipiensis*. *Wolbachia* hinders arbovirus multiplication within mosquitoes, lowering the risk of transmission to humans. Additionally, when *Wolbachia*-infected males mate with uninfected females, the eggs do not develop into viable offspring. NIR spectroscopy can track changes in the spread of arboviruses after the release of *Wolbachia*, allowing health authorities to efficiently and inexpensively monitor the effectiveness of *Wolbachia* in reducing disease transmission and preventing outbreaks. To assess the accuracy and potential of NIR spectroscopy as a cost-effective and rapid tool for arbovirus surveillance, Santos et al. conducted a study using NIR spectroscopy to detect Zika, chikungunya, and *Wolbachia* infections, as well as to determine the post-death age of female *A. aegypti* mosquitoes over seven days. Several studies have analyzed the chemical composition of insect samples

using NIR spectroscopy to detect *Wolbachia* infections. One study evaluated the effectiveness of NIR spectroscopy for identifying species, gender, age, and *Wolbachia* infection in laboratory-reared *Drosophila*, achieving 90% accuracy in detecting *Wolbachia* infections. These results suggest that NIR spectroscopy can potentially identify species, gender, and the presence of *Wolbachia* in fly populations. The findings make NIR spectroscopy a valuable tool for assessing the success of *Wolbachia*-based disease control programs and understanding critical factors like insect age, which is important for evaluating disease transmission risk. Overall, NIR spectroscopy can significantly enhance the efficiency of controlling arbovirus infection outbreaks.

In a separate study, NIR spectroscopy was evaluated for detecting HIV-1 infection without the use of chemical reagents. Plasma samples from individuals infected with HIV-1 and uninfected controls were analyzed. NIR spectra in the 600-1000 nm range were collected and analyzed using PLSR and leave-one-out cross-validation to develop a model for estimating HIV-1 concentration. The same plasma samples were also tested for the HIV-1 p24 antigen using ELISA. The results from the NIR spectroscopic model showed a strong correlation with the HIV-1 p24 ELISA results, indicating that NIR spectroscopy could serve as a rapid, accurate, and cost-effective method for large-scale HIV-1 diagnosis [19].

Additionally, Vis-NIR spectroscopy was utilized to differentiate inflammatory responses caused by influenza A and B virus infections. By examining changes in the water peak spectra in the nasal mucosal regions of infected patients, researchers found that the influenza B virus induces a more severe inflammatory response than the influenza A virus. This distinction is crucial

for enhancing diagnostic accuracy and specificity for these infections. The study suggests that Vis-NIR spectroscopy could also be applied to diagnose other respiratory infections, such as those caused by respiratory adenoviruses and syncytial virus, highlighting its broad potential in medical diagnostics.

A study investigated the use of NIR spectroscopy combined with artificial intelligence to detect Hepatitis C Virus (HCV) in human blood serum. NIR spectroscopy creates a detailed global molecular fingerprint of serum, revealing how the presence of HCV alters the absorbance spectrum and affects the water matrix and key biomolecules such as serum albumin and fatty acids, which are potential biomarkers for HCV. The analysis employed advanced statistical methods, including L1-penalized logistic regression and PCA, to identify significant spectral differences between HCV-positive and negative samples. These machine learning approaches successfully developed a predictive model for HCV detection, indicating that NIR spectroscopy, combined with computational tools, could provide a rapid and accurate method for diagnosing HCV [20].

Bacterial Infections

Spectroscopic techniques for identifying clinical bacteria are an emerging diagnostic method in medicine, although they are already widely used in the food industry. Numerous studies have investigated the potential of near-infrared (NIR) spectroscopy for detecting and distinguishing between different pathogenic bacterial species by analyzing the unique biochemical compositions of bacterial cells. This method relies on the vibrational transitions of various molecular bonds within specific chemical components of the cells, observable in the NIR region, which provides a distinctive spectral pattern for

each bacterial species [21]. The resulting spectra reveal information about various cellular components, including lipids, proteins, nucleic acids, and polysaccharides. Specific absorption bands can be correlated with functional groups such as C-H, O-H, and N-H, indicating the presence and concentration of these molecules. For example, C-H stretching modes correspond to lipids and certain proteins, while O-H groups are typically associated with polysaccharides and water content.

One study focused on detecting and identifying bacterial contamination in liquids using Fourier-transform NIR spectroscopy combined with multivariate pattern recognition. It included bacterial suspensions of *Escherichia coli* HB101, *E. coli* ATCC 43888, *E. coli* 1224, *Bacillus amyloliquifaciens*, *Pseudomonas aeruginosa*, *Listeria innocua*, and *Bacillus cereus*. This method effectively distinguished these strains within the 6000–4000 cm^{-1} spectral region, allowing for rapid and reliable identification with minimal sample preparation. The FT-NIR spectra of different bacterial strains exhibited similar overall patterns, with some bands corresponding to N-acetylmuramic acid, a component of bacterial cell walls. To enhance differentiation and reduce noise, mathematical transformations—specifically the Savitzky-Golay second derivative—were applied [22].

Recent research has provided a practical solution for addressing antimicrobial resistance associated with Carbapenem-Resistant Enterobacteriaceae. This study explored the potential of NIR spectroscopy to identify and differentiate between strains of *E. coli* and *Klebsiella pneumoniae*, including the ability to distinguish carbapenem-resistant strains from susceptible ones. Utilizing genetic

algorithms, the Successive Projection Algorithm combined with Linear Discriminant Analysis, and another model using Genetic Algorithm with Linear Discriminant Analysis, the research demonstrated the feasibility of NIR spectroscopy for rapid bacterial differentiation [23].

In a study an innovative approach was developed to detect tuberculosis using IR and NIR spectroscopy. This research analyzed and compared the spectral signatures of *Mycobacterium tuberculosis*, the causative agent of TB, with those of other bacteria such as *E. coli*, *Mycobacterium smegmatis*, and BCG. By examining variations in cellular composition through Principal Component Analysis, the study illustrated that this method serves as a viable alternative to traditional chemical-based techniques, which are often slower and less precise.

NIR spectroscopy has emerged as a promising tool for detecting infectious diseases in domestic animals by analyzing the biochemical composition of tissues, blood, or other bodily fluids. Yin et al. introduced a novel technique that combines NIR spectroscopy with support vector machine (SVM) algorithms to quickly and accurately identify bacterial species, including *S. aureus* and *E. coli* in infected pig wounds. This technique can detect bacteria in less than five seconds, demonstrating its potential as a non-contact, highly sensitive method for rapid microbial identification, making it a valuable resource for diagnosing infected wounds.

The potential of NIR spectroscopy and aquaphotomics as non-destructive alternatives for diagnosing *Paratuberculosis*, a chronic condition threatening the genetic and economic health of dairy herds caused by *Mycobacterium avium* subspecies *paratuberculosis*. The research investigated how these techniques

can detect changes in biofluids, offering a more efficient, accurate, and non-invasive method for managing Paratuberculosis in dairy cattle [24].

Another study employed NIR spectroscopy across the entire NIR region (700–2495 nm) to identify and classify suspensions of *Lactococcus lactis*, *Listeria innocua* FH, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas mendocina*. The raw spectral data were processed using the Savitzky-Golay algorithm, with analytical techniques such as principal component analysis, partial least squares discriminant analysis, and soft independent modeling of class analogy applied. The results indicated that NIR spectroscopy is a promising method for the rapid and noninvasive identification of these bacterial species.

Another study utilized NIR spectroscopy to diagnose bovine respiratory disease, a leading cause of pneumonia in cattle due to *Mannheimia haemolytica*, a Gram-negative bacterium. This research analyzed the NIR spectral profiles of blood plasma from dairy calves infected with *M. haemolytica*, collected over four days prior (baseline) and 23 days after a controlled intra-bronchial challenge. The spectral profiles, assessed using PCA–LDA models, effectively differentiated between baseline and infected states, indicating that NIR spectroscopy could serve as a valuable tool for monitoring the progression of bovine respiratory disease by capturing physiological and biochemical changes in the bloodstream during infection.

Parasitic Infections

NIR spectroscopy is being investigated as a noninvasive method for detecting parasitic infections through the analysis of changes in blood or serum samples. This technique measures the absorption of near-infrared light by various molecules, producing unique spectral patterns that indicate the presence of parasites. These patterns reflect

alterations in proteins, lipids, and water content due to the infection. Researchers are leveraging NIR spectroscopy data in conjunction with machine learning to create accurate diagnostic methods for parasitic infections.

Numerous studies have assessed NIR spectroscopy as a rapid and cost-effective diagnostic tool for malaria detection. One study examined its potential for identifying malaria, both *in vitro* using cultures of *Plasmodium falciparum* and *in vivo* with *P. berghei*-infected mice. The combination of NIR spectroscopy and machine learning successfully detected and differentiated malaria parasite stages, even at very low levels of parasitemia. The research indicated that NIR spectroscopy could identify malaria byproducts, such as hemozoin, rather than the parasites themselves, enabling highly sensitive detection and providing a rapid diagnostic option for malaria elimination efforts.

Another study evaluated the ability of NIR spectroscopy to detect *Plasmodium falciparum* in *Anopheles coluzzii* mosquitoes. While it performed moderately well with lab-infected mosquitoes, it struggled to identify oocysts or sporozoites in field-caught specimens. Models trained on field mosquitoes did not accurately predict infections in other field mosquitoes, although slightly improved accuracy was observed when focusing on mosquitoes with high parasite loads. Overall, the effectiveness of NIR spectroscopy for detecting malaria in mosquitoes appears limited, particularly in variable field conditions [25].

In a separate study 750 *Anopheles gambiae* mosquitoes were fed *Plasmodium falciparum* and monitored for the development of oocysts and sporozoites. These mosquitoes were then scanned using NIR spectroscopy and analyzed via qPCR for infection detection. The results showed

that NIR spectroscopy could accurately identify oocyst and sporozoite infections with 88% and 95% accuracy, respectively, highlighting its potential as a rapid and cost-effective screening tool for malaria in mosquitoes.

Chagas disease, caused by the parasite *Trypanosoma cruzi*, poses a significant threat to 70 million people worldwide. A study explored the use of near-infrared (NIR) spectroscopy as a rapid, noninvasive method for detecting *T. cruzi* in *Triatoma infestans* vectors. This marks the first application of NIR spectroscopy for identifying *T. cruzi* in triatomine insects. The results demonstrated that NIR spectroscopy could accurately detect *T. cruzi* infections in the midgut and rectum of the insects, as well as in their wet and dry excreta, achieving 100% accuracy. This research highlights NIR spectroscopy as a promising tool for rapid and cost-effective monitoring of Chagas disease, although further studies are necessary to validate its effectiveness in natural environments.

Additionally, a study explored the application of NIR spectroscopy combined with artificial neural networks to detect infections of *Trichuris muris* in mice, which serves as a model for the human whipworm parasite, *Trichuris trichiura*, a common soil-transmitted helminth [26]. This approach analyzes how NIR light is absorbed by various molecules, revealing changes in immune response and tissue alterations due to infection. The study found that NIR spectroscopy, paired with ANN, could effectively distinguish between infected and uninfected mice as early as two weeks post-infection using fecal, blood, and serum samples, as well as through noninvasive skin scans. This method demonstrated advantages in speed, affordability, and reduced training requirements compared to traditional diagnostic techniques like Kato-Katz and

PCR, making it a valuable tool for public health efforts in endemic regions.

Heavy infestations of *Haemonchus contortus*, a blood-feeding parasitic nematode, can lead to severe anemia in sheep, resulting in blood in the feces and various health complications. Early and accurate detection of these infections is crucial for effective treatment and to mitigate their negative impact on sheep health and productivity. The use of portable visible-near-infrared spectroscopy was assessed for detecting *H. contortus* infections in sheep by measuring hemoglobin levels in their feces. Calibration models developed using partial least squares regression and support vector machine regression provided accurate quantification of hemoglobin, with SVMR yielding superior results. Both models demonstrated high sensitivity and specificity for detecting hemoglobin, making Vis-NIR spectroscopy a promising tool for on-farm diagnosis of *H. contortus* infections.

Many studies have reported their performance using various evaluation metrics, complicating direct comparisons. In some instances, commonly expected measures such as accuracy, sensitivity, specificity, or correlation coefficients were omitted. Despite this variability, the studies still illustrate the potential and reliability of NIR spectroscopy for infectious disease diagnostics. This inconsistency in reporting represents a limitation in the current literature and underscores the need for standardized performance metrics in future research.

LIMITATIONS ASSOCIATED WITH NIR SPECTROSCOPY

NIR spectroscopy is a powerful tool for diagnosing infectious diseases, offering advantages such as non-invasive measurement and rapid analysis. However, it also has notable limitations that must be

considered. A primary limitation is the complex nature of biological tissues, which can result in overlapping NIR spectral features. NIR spectroscopy primarily detects molecular vibrations of water, hemoglobin, and other common tissue components found in biological samples. This focus may limit its sensitivity to specific biomarkers associated with particular pathogens, making it challenging to differentiate between various infections. The sensitivity of NIR spectroscopy to the overall composition of samples complicates the analysis of complex biological entities, such as entire organisms or organs. The spectral data can become intricate due to overlapping signals from different components, making it difficult to isolate and identify specific biomarkers, particularly when they are present in low concentrations or localized within specific tissues. For instance, in studies diagnosing mosquito-borne arboviruses using NIR spectroscopy, spectra were collected from the heads and thoraces of mosquitoes to avoid interference from abdominal contents. This approach may have impacted the accuracy of detecting certain infections, such as *Wolbachia* in *A. aegypti*, which is more densely concentrated in the ovaries. This raises an important issue in clinical settings: if a disease or infection is localized in specific areas of the body, scanning only other regions may yield inaccurate results, complicating diagnosis and hindering the identification of the condition [27].

The sensitivity of NIR spectroscopy to water content can be both beneficial and detrimental. In one study, researchers used NIR spectroscopy to differentiate between healthy cattle and those infected with *Paratuberculosis* by analyzing blood plasma. The study showed high sensitivity and specificity for diagnosing *Paratuberculosis*, largely due to changes in water absorbance caused by the infection.

Similarly, another study utilized shifts in the water peak in NIR spectroscopy resulting from temperature changes due to infection to distinguish between influenza A and B. The increase in body temperature is a common response to infection or inflammation and is not unique to influenza, as many infectious or inflammatory conditions can induce such changes. Therefore, in both studies, the ability to differentiate was primarily based on alterations in overall water absorbance rather than the identification of specific biomarkers directly linked to the pathogens. This method may not be applicable to other infections where physiological changes are less pronounced or where specific pathogen-related biomarkers are critical for diagnosis. Additionally, if a different infection caused similar alterations in water content or other general blood plasma components, NIR spectroscopy might struggle to differentiate between the two conditions. This lack of specificity could lead to misclassifications or necessitate the use of additional diagnostic tools to accurately determine the nature of the infection.

Interference from water absorption is a significant limitation of NIR spectroscopy in diagnosing infectious diseases. Water exhibits strong absorption in the NIR region, which can dominate the spectra and obscure changes associated with the presence of pathogens, particularly in biological samples where water content is high. For example, in a study aimed at diagnosing HIV-1 in human plasma, specific wavelengths (e.g., around 970 nm and in the 600–700 nm region) showed strong water absorption that interfered with the detection of HIV-1-specific signals. This interference complicates spectral interpretation and undermines model accuracy.

Differentiating between infections can also be challenging due to overlapping spectra, especially when causative agents share close genetic relationships. For instance, one study reported a sensitivity of only 84% for Zika virus infections compared to 100% for Dengue virus infections. Some Zika-infected mosquitoes were misclassified as Dengue-infected, likely due to similar spectral features and the close genetic relationship between the two viruses.

To address the challenges of spectral interpretation, NIR spectroscopy data often requires extensive preprocessing and advanced chemometric analysis. Studies have emphasized the need for sophisticated algorithms and models to extract meaningful information from NIR spectra when diagnosing infectious diseases. For example, while principal component analysis and support vector machines effectively identified microbes in pig wounds using NIR spectroscopy, further research is necessary to explore improved data processing methods and model development techniques. This would enhance the handling of larger datasets and increase the robustness of NIR spectroscopic models. Another study that detected malaria parasites using NIR spectroscopy and machine learning found that although artificial neural networks showed promise, further optimization of these models is essential to enhance their robustness and accuracy as diagnostic tools. Without such advancements, NIR spectroscopy may struggle with larger, more diverse datasets. This need for advanced data analysis tools poses a significant limitation, particularly in settings with limited computational resources.

CONCLUSION

NIR spectroscopy has emerged as a valuable and versatile technique for the

rapid and efficient diagnosis of infectious diseases in both humans and animals. By analyzing the absorption of specific near-infrared wavelengths by molecular components within biological samples, NIR spectroscopy can provide detailed insights often overlooked by traditional diagnostic methods. Recent advancements in this field, including the development of portable NIR spectroscopy devices and the integration of advanced data analysis techniques, have significantly enhanced its diagnostic capabilities, positioning it as a promising alternative to more complex, time-consuming, and costly molecular diagnostic techniques. Finally, there is a broad consensus across the literature that, in its current state of development, NIR should be primarily employed as a screening tool rather than a definitive diagnostic method.

Author(s) Contribution: resources data, M. Yousaf; proof-reading, G. Murtaza.

Funding: No funding involved.

Ethical Approval: Not applicable

Conflict of Interest: Nil.

Consent for Publication: All authors approved the manuscript for publication.

REFERENCES

1. Mizusawa, M. Updates on Rapid Diagnostic Tests in Infectious Diseases. *Mo. Med.* 2020, 117, 328–337.
2. Srivastava, S.; Singh, P. K.; Vatsalya, V.; Karch, R. C. Developments in the Diagnostic Techniques of Infectious Diseases: Rural and Urban Prospective. *Adv. Infect. Dis.* 2018, 8, 121–138. DOI: 10.4236/aid.2018.83012.
3. Sahu, R. K.; Mordechai, S. Spectroscopic Techniques in Medicine: The Future of Diagnostics. *Appl. Spectrosc. Rev.* 2016, 51, 484–499. DOI: 10.1080/05704928.2016.1157809.
4. Fakayode, S. O.; Baker, G. A.; Bwambok, D. K.; Bhawawet, N.; Elzey, B.; Siraj, N.; Macchi, S.; Pollard, D. A.; Perez, R. L.; Duncan, A. V.; Warner, I. M. Molecular (Raman, NIR, and FTIR) Spectroscopy and Multivariate Analysis in Consumable Products analysis1. *Appl. Spectrosc. Rev.* 2020, 55, 647–723. DOI: 10.1080/05704928.2019.1631176.
5. Beć, K. B.; Grabska, J.; Huck, C. W. Near-Infrared Spectroscopy in Bio-Applications. *Molecules* 2020, 25, 2948. DOI: 10.3390/molecules25122948.
6. Beć, K. B.; Grabska, J.; Huck, C. W. Principles and Applications of Miniaturized Near-Infrared (NIR) Spectrometers. *Chemistry* 2021, 27, 1514–1532. DOI: 10.1002/chem.202002838.
7. Ozaki, Y.; Huck, C. W.; Ishigaki, M.; Ishikawa, D.; Ikehata, A.; Shinzawa, H. Near-Infrared Spectroscopy in Biological Molecules and Tissues. Springer eBooks: Berlin, Heidelberg, 2018, pp 1–19. DOI: 10.1007/978-3-642-35943-9_138-1.
8. Sakudo, A. Near-Infrared Spectroscopy for Medical Applications: Current Status and Future Perspectives. *Clin. Chim. Acta.* 2016, 455, 181–188. DOI: 10.1016/j.cca.2016.02.009.
9. Sikulu-Lord, M. T.; Maia, M. F.; Milali, M. P.; Henry, M.; Mkandawile, G.; Kho, E. A.; Wirtz, R. A.; Hugo, L. E.; Dowell, F. E.; Devine, G. J. Rapid and Non-Destructive Detection and Identification of Two Strains of Wolbachia in Aedes Aegypti by Near Infrared Spectroscopy. *PLoS Negl. Trop. Dis.* 2016, 10, e0004759–e0004759. DOI: 10.1371/journal.pntd.0004759.
10. Sakudo, A.; Suganuma, Y.; Kobayashi, T.; Onodera, T.; Ikuta, K. Near-Infrared Spectroscopy: Promising Diagnostic Tool for Viral Infections. *Biochem.*

- Biophys. Res. Commun. 2006, 341, 279–284. DOI: 10.1016/j.bbrc.2005.12.153.
11. Maia, M. F.; Kapulu, M. C.; Muthui, M. K.; Wagah, M. G.; Ferguson, H. J.; Dowell, F. E.; Baldini, F.; Ranford-Cartwright, L. C. Detection of Plasmodium Falciparum Infected Anopheles Gambiae Using Near-Infrared Spectroscopy. *Malar. J.* 2019, 18, 137. DOI: 10.1186/s12936-019-2719-9.
 12. Kariyawasam, T. N.; Ciocchetta, S.; Visendi, P.; Soares, R. J.; Smith, M. E.; Giacomini, P. R.; Sikulu-Lord, M. T. Near-Infrared Spectroscopy and Machine Learning Algorithms for Rapid and Non-Invasive Detection of Trichuris. *PLoS Negl. Trop. Dis.* 2023, 17, e0011695–e0011695. DOI: 10.1371/journal.pntd.0011695.
 13. Vitorino, R.; Barros, A. S.; Guedes, S.; Caixeta, D. C.; Sabino-Silva, R. Diagnostic and Monitoring Applications Using near Infrared (NIR) Spectroscopy in Cancer and Other Diseases. *Photodiagnosis Photodyn. Ther.* 2023, 42, 103633. DOI: 10.1016/j.pdpdt.2023.103633.
 14. Beć, K. B.; Huck, C. W. Breakthrough Potential in Near-Infrared Spectroscopy: Spectra Simulation. A Review of Recent Developments. *Front. Chem.* 2019, 7, 48. DOI: 10.3389/fchem.2019.00048.
 15. Sandorfy, C.; Buchet, R.; Lachenal, G. Principles of Molecular Vibrations for Near-Infrared Spectroscopy. In *Near-Infrared Spectroscopy in Food Science and Technology*; Ozaki, Y., McClure, F., Christy, A., Eds.; John Wiley & Sons, Inc: Hoboken, NJ, 2007; pp. 11–40.
 16. Roggo, Y.; Chaluz, P.; Maurer, L.; Lema-Martinez, C.; Edmond, A.; Jent, N. A Review of near Infrared Spectroscopy and Chemometrics in Pharmaceutical Technologies. *J. Pharm. Biomed. Anal.* 2007, 44, 683–700. DOI: 10.1016/j.jpba.2007.03.023.
 17. Hibbert, D. B. Vocabulary of Concepts and Terms in Chemometrics (IUPAC Recommendations 2016). *Pure and Applied Chemistry* 2016, 88, 407–443. DOI: 10.1515/pac-2015-0605.
 18. Raposo-Neto, J. J.; Kowalski-Neto, E.; Luiz, W. B.; Fonseca, E. A.; Cedro, A. K. C. L.; Singh, M. N.; Martin, F. L.; Vassallo, P. F.; Campos, L. C. G.; Barauna, V. G. Near-Infrared Spectroscopy with Supervised Machine Learning as a Screening Tool for Neutropenia. *J. Pers. Med.* 2023, 14, 9–9. DOI: 10.3390/jpm14010009.
 19. Wold, S.; Sjöström, M.; Eriksson, L. PLS-Regression: A Basic Tool of Chemometrics. *Chemometrics and Intelligent Laboratory Systems* 2001, 58, 109–130. DOI: 10.1016/S0169-7439(01)00155-1.
 20. Nørgaard, L.; Saudland, A.; Wagner, J.; Nielsen, J. P.; Munck, L.; Engelsen, S. B. Interval Partial Least-Squares Regression (iPLS): A Comparative Chemometric Study with an Example from near-Infrared Spectroscopy. *Appl. Spectrosc.* 2000, 54, 413–419. DOI: 10.1366/0003702001949500.
 21. Araújo, M. C. U.; Saldanha, T. C. B.; Galvão, R. K. H.; Yoneyama, T.; Chame, H. C.; Visani, V. The Successive Projections Algorithm for Variable Selection in Spectroscopic Multivariate Calibration. *Chemometr. Intell. Lab. Syst.* 2001, 57, 65–73. DOI: 10.1016/S0169-7439(01)00119-8.
 22. Li, H.; Liang, Y.; Xu, Q.; Cao, D. Key Wavelengths Screening Using Competitive Adaptive Reweighted Sampling (CARS) for Multivariate Calibration. *Anal. Chim. Acta.* 2009,

- 648, 77–84. DOI: 10.1016/j.aca.2009.06.046.
23. Yang, J.; Xu, J.; Zhang, X.; Wu, C.; Lin, T.; Ying, Y. Deep Learning for Vibrational Spectral Analysis: Recent Progress and a Practical Guide. *Anal. Chim. Acta.* 2019, 1081, 6–17. DOI: 10.1016/j.aca.2019.06.012.
 24. The Effects of Sample Presentation in Near-Infrared (NIR) Spectroscopy;. VIAVI Solutions Inc: Chandler, Arizona, U.S, 2021.
 25. Yin, M.; Li, J.; Huang, L.; Li, Y.; Yuan, M.; Luo, Y.; Armato, U.; Zhang, L.; Wei, Y.; Li, Y.; et al. Identification of Microbes in Wounds via Near-Infrared Spectroscopy. *Burns* 2022, 48, 791–798. DOI: 10.1016/j.burns.2021.09.002.
 26. Strangman, G.; Franceschini, M. A.; Boas, D. A. Factors Affecting the Accuracy of Near-Infrared Spectroscopy Concentration Calculations for Focal Changes in Oxygenation Parameters. *Neuroimage.* 2003, 18, 865–879. DOI: 10.1016/s1053-8119(03)00021-1.
 27. Tripathi, P.; Harsh. Medical Viruses: Diagnostic Techniques. *Virol J*, 1432023, 20 (1). DOI: 10.1186/s12985-023-02108-w.