INFORMATION BRIEF (RAPID REVIEW)

Non-Invasive Fourier Transform Infrared (FTIR) Quantum Cascade Laser Spectroscopy

Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia
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Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia
Level 4, Block E1, Precinct 1
Government Office Complex
62590, Putrajaya
Tel: 603 8883 1229

Available online via the official Ministry of Health Malaysia website: http://www.moh.gov.my

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TITLE: NON-INVASIVE FOURNIER TRANSFORM INFRARED (FTIR) QUANTUM CASCADE LASER SPECTROSCOPY

PURPOSE

To provide brief information on the effectiveness, safety and cost-effectiveness of non-invasive Fournier Transform Infrared (FTIR) Quantum Cascade Laser (QCL) Spectroscopy to analyse human blood following a request from the Director General of Health office, Ministry of Health, Malaysia.

BACKGROUND

Fournier Transform Infrared (FTIR) is widely used in many industries and is used for the analysis of both organic and inorganic compounds. It can confirm the composition of both solids, liquids, and gases. This technique is mainly used for the identification of unknown compounds and it can utilises mid-infrared light, which interacts with molecular vibrations in the sample, providing a detailed chemical fingerprint. This spectroscopy is used to generate bacterial spectral scans based on the molecular composition of a sample. Basically, infrared spectroscopy consists of the infrared source, the sample, and the detector. (FTIR) spectroscopy is an analytical technique used to identify and quantify the chemical composition of a sample such as structure and dynamics of polypeptides and proteins. Vibrations of the polypeptide repeat units of proteins result in nine characteristic group frequencies in the mid-IR region referred to as amide bands. Furthermore, FTIR spectroscopy has been used in the analysis and identification of functional groups of various materials, including polysaccharides in plant materials. The identification of the functional groups provides information about the chemical composition, molecular conformation, and hydrogen bonding patterns. Additionally, Fourier transform infrared spectroscopy has been used for preliminary analysis of the chemical composition of wastewater and algal biomass.[1]
Diagnostic devices currently available on the market rely on the same measuring techniques developed in the last century (mainly spectrophotometry or electrochemical assays). Meanwhile, viruses, bacteria, and fungi are rapidly evolving, pushing further the need to develop new, quick, and reliable diagnostic tools. The primary, commercially available measuring techniques for such devices are spectrophotometry, enzyme-linked immunosorbent assay (ELISA), electrophoresis, and blood cell counting or complete blood count (CBC). However, all of these methods have limitations. In ultraviolet–visible (UV–VIS) spectrophotometry, the main limitation is the requirement for sample and setup preparation time to avoid light interferences. ELISA limitations are related to the cost of the assays due to the use of antibodies, the risk of cross-reactivity, the high background noise, and extended analysis time. Electrophoresis requires a large sample for the assays, as well as high analysis precision. Lastly, CBC limitations are related to the manual examination of blood smears, difficulty recognising abnormal red cell shapes (such as fragmented cells), and high running costs. Hence, the pressing need for new, fast, and precise analysing techniques.[1,3]

TECHNICAL FEATURES

Unlike traditional blood tests that require blood draws, FTIR-QCL can analyse blood through the skin, reducing discomfort and risk of infection. The analysis may be performed faster and often in real-time, which is advantageous for rapid diagnostics. The technique typically requires little to no sample preparation, making it more convenient and reducing the potential for sample contamination or degradation. The technique, (FTIR-QCL) may provide quantitative information about the concentration of various blood components, including glucose, proteins, and lipids.[1,2] The aim of spectroscopy techniques (FTIR or bright perceptible (UV–Vis) spectroscopy) is to quantify how much light a sample absorbs at each frequency.

The method is based on the vibrations of atom molecules that modify the dipolar moment of the molecule and chemical bonds have specific frequencies of vibration that can be detected using FTIR. Infrared Region (IR) radiation is a group of electromagnetic waves (EMR) with wavelengths longer than visible radiation, invisible to the human eye. The IR region of the electromagnetic spectrum ranges in wavelengths from 0.8–100 μm, as shown in **Table 1**. Typically, the IR is broken into three ranges, near-IR, mid-IR, and far-IR. Most of the IR used in medical applications are in the mid-IR range, considering radiation from the electromagnetic spectrum, in the wavenumber interval from 4000 cm^{-1} to 400 cm^{-1}. The frequency of the absorbed radiation is responsible for each subatomic vibrational interaction, as seen in **Figure 1**.

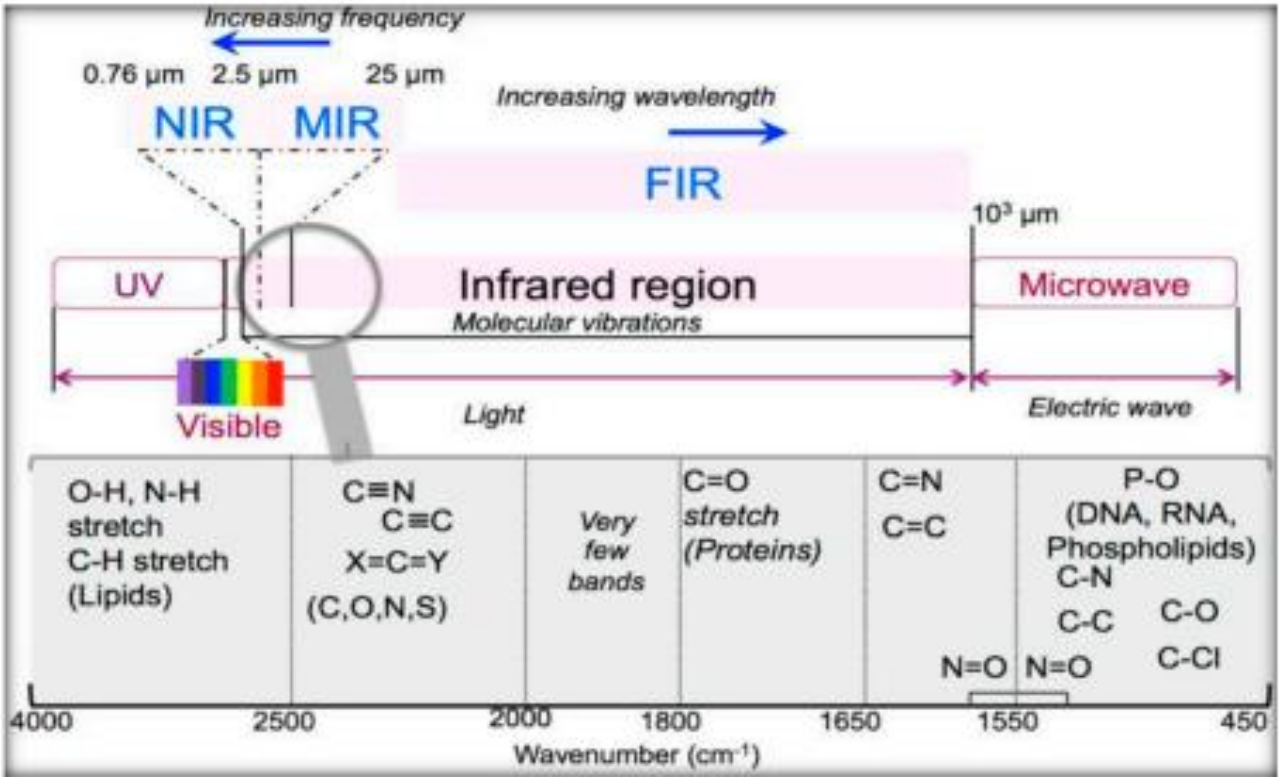

Figure 1: Scheme of the optical spectrum, focusing on the infrared region

Table 1: Infra-Red Regions

Region	Wavelength (µm)	Wavenumbers (cm^{-1})	Frequency ($x10^{14}$ Hz)
Near-IR	0.8 – 2.5	12,500 - 4000	3.75 – 1.2
Mid-IR	2.5 – 25	4000 - 400	1.2 – 0.12
Far-IR	25 – 100	400 -100	0.12 – 0.03
Frequently Used	**2.5 - 15**	**400 -670**	**1.2 – 0.20**

The main limitations of FTIR spectroscopy relate to the tissue depth penetration of the infrared light, which only allows biochemical analysis of the tissues up to a few dozens of micrometres. Additionally, in the conventional FTIR spectroscopy, which works in transmission mode and consequently with no incidence angle between emitter and sample, there is difficulty in assuring the reproducibility of the spacer thickness when using liquid samples.[1]

EVIDENCE SUMMARY

A comprehensive electronic search was performed and a systematic search was conducted using the scientific databases via OVID, PubMed and general search engines [Google Scholar], using the search term; *Fournier Transform Infrared (FTIR), Quantum Cascade Laser (QCL), "FTIR", "spectroscopy", "optics", "infrared" and "blood, blood cells".* The last search was conducted on 1 December 2023. The is one literature review was found to be relevant and included in this review.

Fadlelmoula A (2022) conducted a review on *Fourier Transform Infrared (FTIR) Spectroscopy to Analyse Human Blood over the Last 20 Years: A Review towards Lab-on-a-Chip Devices* aimed to describes the main concepts related to FTIR and presents the latest research focusing on FTIR spectroscopy technology and its integration in lab-on-a-chip devices and their applications in the biological field. The relevant studies resulting from the database search were manually analysed to identify other potential studies to be included. The exclusion criteria were: reviews, comments, overviews, case reports, viewpoints and perspectives, as well as documents reporting tests with data ambiguity. Studies not written in the English language were also excluded, as well as duplicate results.[1]

Among the different spectroscopic techniques develop to distinguish between normal to cancerous blood tissues, Fourier transformed spectroscopy has shown some potential. The biomedicine's IR-based techniques have become a reality with a large amount of information accumulated from clinical studies, trials, and developments. In 2013, FTIR spectroscopy was applied to study healthy and cancerous blood samples, using a diffuse reflectance technique from SHIMADZU 8000 series FTIR spectrophotometer. The spectra of cancerous and healthy blood were registered at a resolution of 4 cm^{-1} in the region of 900 cm^{-1} to 2000 cm^{-1}, as observed in **Figure 2**. The obtained results show that the bands of proteins, lipids, carbohydrates, and nucleic acids from cancerous samples are clearly different from the normal ones dominated by two absorption bands at 1643 cm^{-1} to 1550 cm^{-1}, known as amide

I and amide II. Amide I appears from the C=O stretching vibrations and amide II from the C–N stretching and CNH bending vibrations.[1]

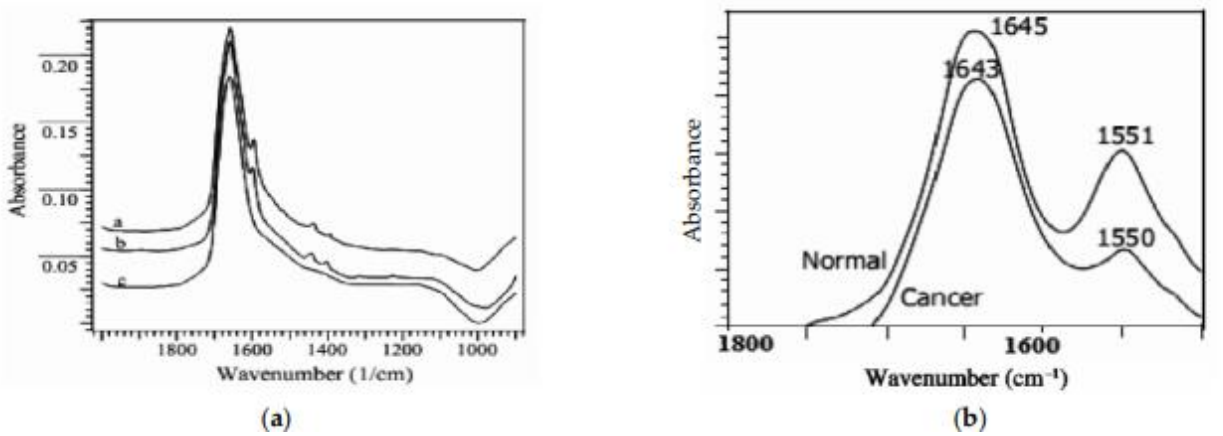

Figure 2: (a) FTIR absorption spectra of 'a' cancerous blood, 'b' normal blood and 'c' water samples using air as a reference; (b) detail of the FTIR absorption spectra of the normal and cancerous blood.

FTIR was also used to detect biomarkers for early screening of pediatric leukaemia. In the reported study, the spectra were acquired from blood serum samples of ten child patients with B-cell precursor lymphoblastic leukaemia (BCP-ALL) and were contrasted with ten control samples. No clear peak shift was spotted between the averaged spectra of leukaemia patients and healthy individuals at the first trial.[1]

In 2020, the attenuated total reflectance Fourier transform (ATR-FTIR) spectroscopy was considered for distinguishing HIV-infected patients from healthy uninfected controls. This study comprised one hundred and twenty blood plasma samples of pregnant women and allowed to obtain good sensitivity (83%) and specificity (95%) using a genetic set of rules with linear discriminant assessment (GA-LDA). In the range of 1800 cm^{-1} to 900 cm^{-1}, the spectra displayed some particular feature absorptions, including the amide I band at 1635 cm^{-1}, an arm at 1560 cm^{-1} (due to C=O, Amide II) and three small depth absorptions at 1480 cm^{-1} (corresponding to the C-H asymmetric deformation of methyl agencies), at 1404 cm−1 (due to the COO−symmetric stretching of proteins and lipids) and 1060 cm^{-1} (due to the C-O nucleic acids). Due to the similarity between the spectral features in the groups (uninfected control and HIV infected), chemometric patterns were used to identify spectral features responsible for class differentiation as seen in **Figure 3**. ATR-FTIR spectroscopy with multivariate analysis was able to accurately identify HIV-infected pregnant women based on blood plasma, showing the potential of this method for early detection of HIV in a fast and reagent-free approach. Successful development of this method in a clinical environment could aid early diagnosis of gestational HIV and help treatment.

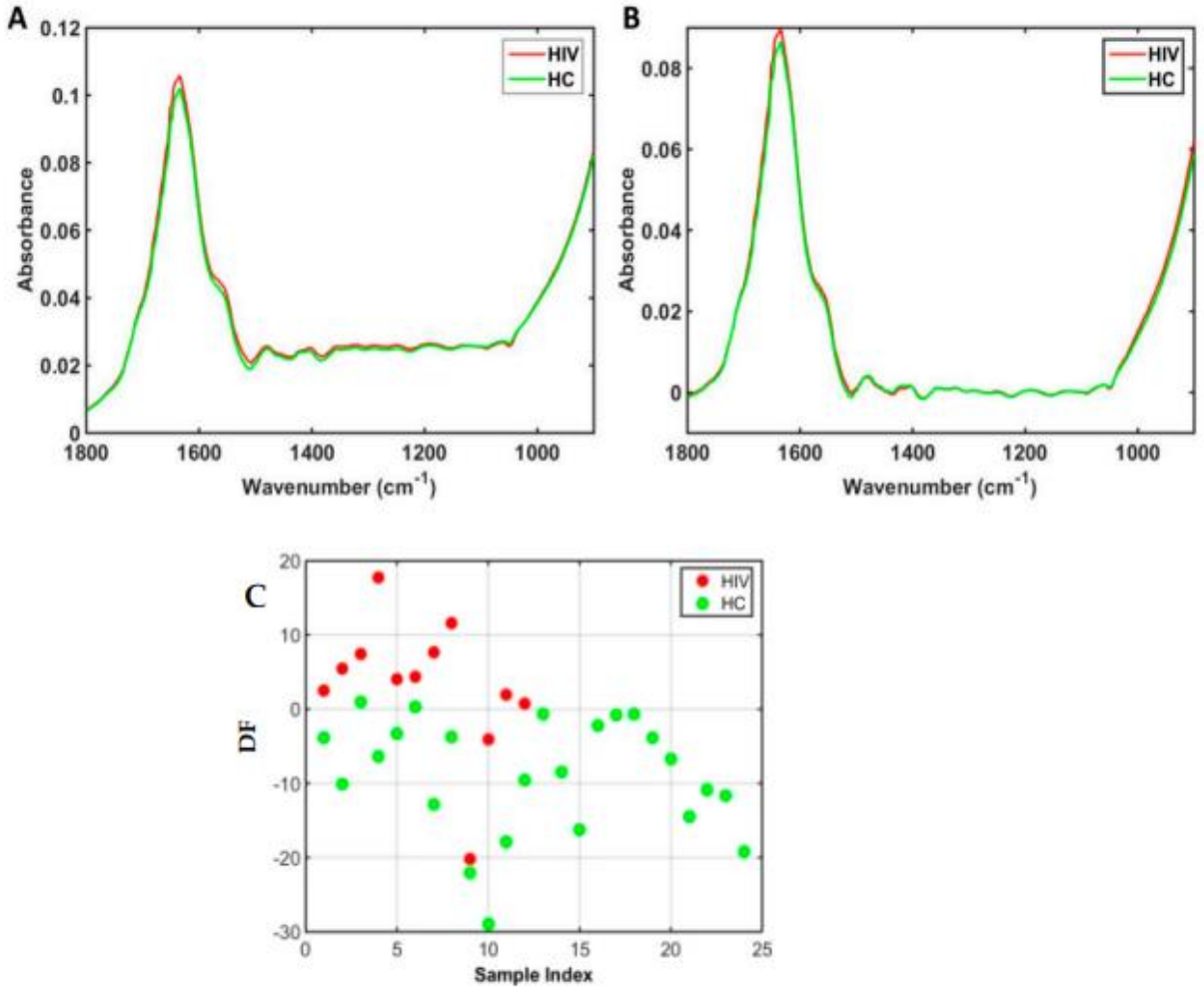

Figure 3: ATR-FTIR spectra for distinguishing between HIV infected and healthy blood samples. (A) Mean raw IR spectra in the bio fingerprint region (1800 cm^{-1} – 900 cm^{-1}) for HIV-infected (HIV) and healthy uninfected controls (HC) samples. (B) Mean preprocessed IR spectra (AWLS baseline correction) in the bio fingerprint region (1800 cm^{-1} – 900 cm^{-1}) for HIV-infected (HIV) and healthy uninfected controls (HC) samples. (C) Discriminant function (DF) for the samples in the test set, where HIV stands for HIV-infected samples and HC for healthy uninfected controls, allowing their distinction.

CONCLUSION

Based on the review, there was very limited evidence or studies to demonstrated the used of Fournier Transform Infrared (FTIR), Quantum Cascade Laser (QCL) for analysing the human blood cells. This review presents the potential use of FTIR to distinguish between healthy and pathological samples, with examples of early cancer detection, human immunodeficiency virus (HIV) detection, and routine blood analysis, among others.

The development of such devices will be a step ahead in state of the art and will overcome the limitations of current technologies. Thus, to achieve such a goal, future works need to consider the design, fabrication, characterisation, and optimisation of laboratory platforms,

with IR radiation and Fourier Transform post-processing, to examine blood cells, distinguishing between normal and pathological ones, and to better understand several mechanisms of treatment resistance and progression.

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Prepared by

Dr. Ana Fizalinda Abdullah Sani
Senior Principal Assistant Director
Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia

Reviewed by

Dr. Izzuna Mudla Mohamed Ghazali
Public Health Physician
Deputy Director
Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia

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