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Review Article

Diagnosis of Infectious Diseases: Tuberculosis, Malaria and HIV and Aids – A Review

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Abstract

Achieving and sustaining global healthcare is one of the challenges of our time. Healthcare entails sensitive and competent diagnosis of pathogens, treatment and prevention. Infectious diseases have claimed millions of lives and escalated poverty conditions especially in developing regions. Hence, the United Nations Millennium Development Goals (MDGs) between the years 2000 and 2015 included healthcare: the eradication of infectious diseases and rehabilitating societal damages triggered by the diseases. Great efforts have been taken towards this goal; evident by the decline in the statistics of global malaria, human immune deficiency virus (HIV) infections and mortality cases recorded at the end of the MDGs era. However, current infectious diseases statistics from resource-constrained regions are still a sore point. Africa and South East Asia recorded the highest number of both new infections and death cases worldwide. Science and technology face the challenge of producing Point of Care Testing (POCT) devices suitable for such regions to achieve efficient detection of diseases across the global spectrum. This paper reviews traditional and new techniques used for the detection of some infectious diseases: malaria, tuberculosis and HIV. The drawbacks of these techniques are discussed and solutions such as Plasmonic metal-based immuno chemical biosensors are considered. For example, vibrational spectroscopy (Raman), which has a potential for the detection and identification of infectious diseases using traditionally weaker intrinsic Raman signals can be improved by the inclusion of the Plasmonic metals, a phenomenon called Surface Enhanced Raman spectroscopy (SERS). Hence, with innumerable references, this paper also reviews SERS application in biosensing and synergising with new technologies such as microfluidics.

Keywords: POCT; TB; malaria; HIV; SERS; Diagnosis

Introduction

Infectious diseases such as malaria, tuberculosis (TB), human immunodeficiency virus (HIV) and cholera, to name a few, continue to cause indisposition and mortality in the world [1]. Infectious diseases account for roughly 16.2 % of the world's mortality and children are the most affected group [2]. Globalisation and travelling catalyses the worldwide spread of these infectious diseases [1]. Hence, early detection is vital to make informed treatment and prevention decisions. Over the years, extensive discoveries on diagnostics have been attained, evident by the vast number of assays at our disposal e.g., gold standard methods: microscopy, tissue culture, lateral flow immunoassays, enzyme-linked immunosorbent assays (ELISA) and recently developed polymerase chain reaction (PCR) [1,3]. Although, a decline in mortality cases has been recorded by the World Health Organisation (WHO), developing regions continue to be burdened [1,3]. Disadvantaged regions cannot afford the expensive and

infrastructural requirements of the available diagnostic assays which explain their continued struggle [1,3]. The high mortality rates experienced in these regions; Sub-Saharan Africa and India, aggravates the standard of living, i.e., increasing numbers of orphans and poverty. The development of sensitive, robust diagnostics for infectious diseases can greatly reduce mortality, encourage better health, and enhance the productivity of the individuals which encourages poverty alleviation [1]. Hence the urgent demand for affordable, sensitive, robust diagnostic tools as an improvement to the traditional diagnostic techniques encouraging their adoption as point of care testing (POCT) devices administered at the bedside in poverty stricken community [1,3]. An ideal POCT tool should be fast, reliable, inexpensive, multiplexing, and portable [4] and is in accordance with the WHO standard, ASSURED (Affordable, Sensitive, Specific, User-friendly, Robust and rapid, Equipment free and Delivered to the patient) [1,3-5]. This review deliberate on the pros and cons of some of the available diagnostic techniques for malaria, TB and HIV.

Malaria

Malaria is caused by Plasmodium genus protozoan. The Plasmodium genus has five species: vivax, knowlesi, malariae, ovale and falciparum, and the latter is the deadliest of the five [6-8]. Malaria is transmitted by female anopheles mosquitoes mostly found in humid areas. It gets injected into the bloodstream (erythrocytes) in the form of sporozoites which then travels and targets the liver where it multiplies asexually in a period of about 7-10 days [9]. At this stage, the infection remains asymptomatic. In the liver, the protozoan can either be stored in a dormant stage which can relapse after several years or the parasite (merozoite) can disseminate from the liver into the bloodstream where it permeates and multiplies in erythrocytes thus causing rupture and reinfection of more erythrocytes [1,6]. Figure 1 further gives an illustration of the malaria life cycle. Malaria presents with flu-like symptoms such as high fever, shaking chills and sweating 10-15 days following infection which makes it hard to rely on these symptoms for early diagnosis. It is then followed up by more aggressive symptoms, some of which are nausea and/or vomiting, diarrhoea, severe headache, fatigue, jaundice and later seizures, confusion and kidney failure. Clinical manifestations of malaria include severe anaemia, thrombocytopenia, hypoglycaemia, metabolic acidosis, hyperlactatemia and others [10,11].

Malaria likely affects anyone exposed to the parasite. However, children and pregnant women are the most affected due to under-developed immune system and lowered immunity, respectively [1]. In such cases, the monocytes produce high levels of cytokines like Tumour Necrosis Factors to regulate the immune response. The immune system produces antibodies against the merozoites or proteins expressed from the infected erythrocyte. The antibodies help in neutralizing the parasite by preventing further infection, limit parasite growth and increase phagocytosis and clearance of the infected cells by macrophages. However, it is a hurdle to develop antimalarial immunity that can completely get rid of the parasite on its own due to the polymorphism of the target malaria antigens [12]. The Plasmodium genome is susceptible to mutations thus diversifies the population on variants [13]. Hence it is important for early diagnosis. Malaria claimed about a million sub-Saharan African and Indian lives in 2010 and continues to claim 548 000 year on year [14]. At the end of the millennium development goals (MDGs) era, 2015, a global decline in malaria cases and mortality across all ages were reported. A 41 % decline was attained between 2000 and 2015 and 21 % from 2010 to 2015. Developing regions showed most malaria prevailing cases at the end of the MDGs; 429 000 global malaria mortality cases surfaced mostly from the WHO African region (92 %), south-east Asia (6 %) and the eastern Mediterranean region (2 %) [15]. The P. falciparum species was responsible for 99 % of the deaths, and 3100 deaths were due to vivax. Commendable strides towards malaria prevention, diagnosis and treatment were taken as confirmed by the global malaria cases and mortality decline. However, the persistent prevalence of cases in developing regions, Africa and South east India is still a major concern. Developing regions suffer from lack of knowledge, poor infrastructure to support available diagnostic devices, and stigmatised views [15]. The current diagnostic tools are not adapted for these regions; hence the urgent need for POCT devices which meet the ASSURED WHO standard [16]. The Malaria global technical strategy (GTS), 2016-2030, focuses on decreasing malaria incidences by 90 % in 2030; eliminating malaria from 35 infected countries; and preventing malaria spread to malaria free regions. The GTS align to the sustainable development goals (SDGs) aiming to eradicate AIDs, TB, malaria and neglected tropical diseases by 2030 [15] (Figure 1).

Malaria diagnosis

Diagnosis and identification of malaria species is paramount to the treatment and attainment of the set envisioned GTS. A gold standard, the "Giemsa-stained blood smear" is used for diagnosis and a series of other techniques ranging from microscopy (fluorescence), spectroscopy (laser desorption mass spectrometry), and molecular (PCR, antigen detecting immunochromatographic strips) [14,18]. Table 1 gives a summary of the various malaria detection assays. Early detection of the causative bacterium can lower mortality, overuse of the malaria drugs and eliminate the issue of drug resistance due to overuse [19]. A number of the economical tools listed in Table 1 show low/poor sensitivity at low parasitemia levels in comparison to their counterparts with enhanced sensitivity, e.g., mass spectroscopy, ATR-IR, and PCR which are not economical. Lateral flow immunochromatographic assays have potential for developing regions although their sensitivity, identification of different strains and co-infections requires improvement [1] (Table 1).

Table 1: Malaria diagnostic assays/tools.

Diagnostic tool techniques	Detection limit	Rapidity	Advantages	Disadvantages
Giemsa-stained blood smear microscopy Two drops of blood from a metal lancet pricked finger are positioned on a glass microscope slide. One drop is smeared into a thin film and the second is not smeared, thick film [20]. The slide is air dried, and the thin film is chemically fixed on the slide. Subsequently, the slide is stained with giemsa solution or an alternative to enhance parasite detection [20]. The water in the solution will lyse unfixed red blood cells from the thick film and remove the haemoglobin. While white blood cells and the parasite remain fixed on the slide. The thick film facilitates detection of the parasite from a large blood volume and the thin film allows identification and quantification of the parasite species [20].	4 to 20 parasites/µl in thick smear [21]	30-60 min [21]	Economical, parasite density analysis, specific to plasmodium species and sexual gametocytes stages [21,22].	Time consuming, requires a trained personnel (5), low sensitivity at low parasitemia [21,23].
Laser desorption mass spectrometry (LDMS) LDMS is used to identify malaria pigment, hemozoin, which can absorb ultraviolet laser. Hemozoin has a heme cation radical and more than five structurally characteristic heme fragments ions. The patient blood is collected and diluted into a heparin phosphate buffer saline (PBS), applied onto metal slides and air-dried before the LDMS and laser exposure [24].	Culture: 10 p/µl Sample: 100 p/µl [24]	<1 min [24]	High throughput, 52% sensitivity, 92 % specificity, parasitemia detected 4-5 days earlier than microscopy [25]	Expensive and intense sample preparation [14]. lack of speciation, impossible to monitor response to treatment due to persisting hemozoin even post treatment [25].
Flow cytometry Infected blood is treated with formaldehyde, which lyses erythrocytes, and preserves parasite and white blood cells. The blood sample is stained with dye and analysed by flow cytometry. The parasite and white blood cells are imaged and counted using blue Hoechst fluorescence and forward scattering [26].		poor	48.6-95 % sensitivity, used for routinely screening of febrile patients in non-endemic regions [25].	Expensive, not reliable to be used as a sole testing technique, inability to quantify or speciate parastemia [25].
Microsphere-based assays Microsphere-based assays are an ensemble of size/fluorescence identified microspheres and pathogen antigen [25]. Each antigen is conjugated to a different microsphere. The main purpose of the system is to detect pathogen-specific antibodies from patient blood. The antigen will capture the antibody and it is detected by the fluorescent conjugate [25]. To attain a positive serology of an antibody, a flow cytometry is used to detect the corresponding microsphere associated with the fluorescent conjugate [25].	100 fg/mL protein	<3	Capacity for sensitivity, quantification, reproducibility, high throughput, analysis of a wide range compounds, and multiplexing [25].	Intense research on development, validation and implementation [25].
Quantitative buffy coat method (QBC) QBC fluorescence microscopy-based technique uses a special galls tubing with a specific internal diameter containing acridine orange stain. The tubing is filled with patient blood, capped and inserted with a small plastic float. About 90 % of the capillary interior is displaced by the float [20]. The float settles at the plasma-red blood cell interface when the tube is centrifuged, and this expands the length of the buffy coat layer by 10-fold. White blood cells will appear as a distinct band and can be quantified by optical device whereas red blood parasite cells due to their lower density will be concentrated in the top portion. And the float will expand and extend to that portion. A fluorescence microscope is used to observe the capillary tube [20].	1000 parasites/µl [14]	<15min	Rapid, improved sensitivity at low parasitemia, simple, reliable, user friendly [21].	Cannot identify different species and parasite load, expensive, compromised specificity due to staining [21].
Immunofluorescence antibody testing (IFA) The serological test detects antibodies against parasite. IFA is the popular reliable serological method and is founded on the production of specific antibodies within 2 weeks of infection which continue to 3-6 months after the parasite clearance. Specific antigens are smeared onto a slide and kept at -30 **°C** before use and they are used to identify and quantify antibodies in a patient sample [21].	Not known	30 -60 min [14]	High sensitivity and specificity, simple [27].	Trained personnel required, costly since it requires fluorescence microscopy, cannot be programmed [14,21].

Attenuated total reflectance infrared spectroscopy (ATR_IR) The patient's blood sample is spun down to separate its components and leukocytes and plasma are removed. The erythrocytes are fixed in methanol and about 5 **µ**L aliquot is placed on the spectrometer diamond, dried, and analysed [23].	0.5 parasite/**µ**l	seconds	Good sensitivity and specificity [23]	Expensive, time consuming sample preparation [14].
Immunochromatographic tests (lateral flow) Monoclonal antibodies are prepared against a malaria antigen target and conjugated to liposome selenium dye or gold in a mobile phase. The mobile phase and its components migrate across a nitrocellulose membrane surface and capture parasite antigen from peripheral blood [28]. The cellulose membrane has capture monoclonal antibody immobile phase, and it captures the labelled antigen, producing a visible coloured line on the strip. The porosity of the membrane and components of the buffer influence the migration rate of the mobile phase [28].	50-100 parasite/**µ**l [14]	10-15 min [14]	Fast, Simple, user friendly with proper training, POCT, moderate sensitivity and specificity, potential for self-test application [25]. Small sample required and multi-strain identification [14].	Lack of sensitivity at low parasite density, false negative results observed for PfHRP2 due to non-specific binding, performance influenced by extreme temperature and humidity [14] Discordant literature reports on specificity and selectivity. Poor speciation and quantification [25].
Polymerase chain reaction (PCR) The identification of parasite through *in-vitro* amplification of specific DNA or nucleic acid sequences [20]. PCR components include the following: DNA polymerase, equal amounts of each of four DNA nucleotide dNTp building blocks, DNA template source, 2 oligonucleotide primers (15-30 bp in length). The amplification of DNA occurs in 3 stages: i) Denaturing – the double-stranded DNA is heated to 90 **°C** for 60 s to separate the strands. ii) Annealing – the temperature is lowered to 35-55 **°C** for 60-120 s to allow binding oligonucleotide primers onto complementary DNA sequence on the single strands. iii) Extension – Temperature is raised to 72 **°C** for 60-180 s to allow enzymatic primer extension reaction. The reaction affords complementary copies of the initial single strands from the primer bound to the DNA. The 3 stages are repeated between 25-40 times [29].	0.004-5 p/**µ**l [14]	Conventional PCR >6h Real time PCT <1h [14]	Conventional PCR: Good sensitivity and specificity, POCT, diagnosis of mixed infections. Real time PCR: multiplexing potential, excellent specificity and selectivity, minimised cross contamination, semi quantitative, possibility to monitor response to treatment [14].	Conventional: Time consuming, complicated sample preparation (6-8 h), cross contamination [25].
Loop-mediated isothermal amplification (LAMP) LAMP, like PCR, uses nucleic acid amplification. The technique was developed by Eiken chemical co, Ltd, and it uses 4 different primers specifically developed to distinguish discrete regions on the target gene. Amplification and detection are achieved in a single step: sample, primer, DNA polymerase with strand displacement activity and subtracts are mixed and incubated at 65 **°C**, resulting in 10^9-10^{10} DNA amplification in 15-60 s [30].	>5 parasite/**µ**l [14]	<1h [14]	POCT mostly eligible for poverty stricken regions, simple, sensitive, specific, semi quantitative, economical [14]	Requires cold storage for reagents, validation of LAMP clinical use is required [25].
Magneto optical technology using polarized light The technique is based on the presence of hemozoin in malaria infected blood. Hemozoin is a detox product of malaria parasite from feeding on globin compound from haemoglobin. Hemozoin is birefringent, paramagnetic and dichroic. The hemozoin compound is aligned into a common axis in the presence of a magnetic field, and subsequently, net dichroism linearly comparable to the concentration of the hemozoin is induced into the sample to enable diagnosis [31-33].	5 ng **µ**L^{-1} or less [34]		Potential for early diagnosis [14]	Vibrating magnets result in signal drift, and limit sensitivity [35].

Nuclear Magnetic resonance (NMR) relaxometry NMR relaxometry studies the characteristic physical and dynamic attributes of a sample by measuring the nuclear spin relaxation time. Hemozoin crystals, present in malaria parasite infected blood, induces changes in water T_2 value of red blood cells and this is used to confirm the infection [36].			Non-invasive, portable, simple economic, rapid and label-free diagnosis [14].	low sensitivity [14]

Table 2: TB diagnostic techniques or assays.

Diagnostic assay	Detection limit	Rapidity	Advantages	Disadvantages or limitations
Chest radiology Pulmonary TB is indicated by a chest X-ray showing lesions in the lungs (perforated upper lobes and segmented lower lobes). However, results cannot be concluded based only on the X-ray because its specificity is not constant and it lacks TB bacterium pathognomonic [45].			Sensitive and useful technique for preliminary screening and narrowing-down possible illness causatives [45]	Low specificity and requires trained personnel [1,45].
Skin patches **Tuberculin skin test (TST)/purified protein derivative (PPD)** A small amount of PDD is injected beneath the skin surface of a patient's inner forearm and left for some hours. After 48-72 hours if the skin is welt free patient is negative and positive if the skin has a welt 10mm [46]. **MPB64 skin patches** MPB64 (immunogenic M. tuberculosis specific antigen) loaded skin patches can detect active TB infection. The MPB64 protein is secreted during active growth of the mycobacterium complex, MTB, M. africanum, M. bovis and M. bovis calmette-Guérin [47,48].		3-4 days [47]	Simple, non-invasive, economical [40] Simple, non-invasive, specific, sensitive, equipment free and does not require trained personnel [47].	Complicated and requires well-trained operators, low accuracy, low sensitivity especially in HIV positive patient due to low immune response [40], requires 2 clinic visitations, give false positive result in patients vaccinated with Bacillus, cannot differentiate latent /active TB [1,46]. Biological mechanism not understood
Interferon-gamma (IFN-γ) release assays (IGRAs) An *in-vitro* technique based on the increased production of IFN-γ from white blood cells previously infected with TB antigens when they are re-exposed to the mycobacterium. The amount of IFN-γ produced is linked to the TB infection. There are 2 commercially available IGRAs assays: (i) QuantiFERON-TB assay – utilises an enzyme-linked immunosorbent assay (ELISA) to measure the T-cell produced IFN-γ. QuantiFERON uses PPD antigen which only gives result for patients previously infected by TB. For new infection cases, QuantiFERON Gold which uses antigens Esat 6, CFP10 and TB 7.7 is suitable. (ii) T SPOT-TB test- Uses an alternative of ELISA, enzyme-linked immunospot and consist of ESAT6 and CFP10 antigens. The IGRAs and TST methods can be used complimentary [45, 46, 49].			Improved specificity [1] Improved accuracy especially with assays using more than one antigen [40].	Failure to distinguish latent and active TB, low sensitivity in HIV infected patients, well-trained operators required, Slow analysis which requires specific instrumentation [40].

Sputum smear microscopy Sputum is collected into special cups. Sputum smears are made onto clean slides, stained by Ziehl-Neelson method and viewed under a fluorescent microscope. One or more presence of acid-fast bacilli is a positive result [50].	≥10,000 bacilli mL^{-1} [40]		Cheaper, fast User-friendly, specific [49].	Low sensitivity especially in low bacterium concentration, to improve sensitivity requires a fluorescence microscope or bleach method. Failure to differentiate mycobacterial strains [40].
Urine based antigen detection assay Strip detects lipoarabinomannan antigen in unprocessed urine samples [46].		25 minutes	Non-invasive, effective in HIV positive people, POCT, economical, moderate training for the personnel [40,46].	Sensitivity dependent on CD4 cell count of HIV patients [40, 46].
Rapid culture systems Identification of MTB strains using indirect drug susceptibility testing (DST) on solid/liquid culture is a gold standard clinical and research TB diagnosis tool. The bacterium is isolated and undergoes DST in the presence of anti TB-drug. Solid cultures require trained personnel and specially equipped laboratory and take long (10-12 weeks) for *M. tuberculosis* to grow. Hence, liquid cultures have replaced the former owing to their improved sensitivity, and enhanced rapidity (2-3 weeks) [46,51,52].		2 weeks [46,52].	Sensitive than sputum smears, specific [46].	Expensive, prone to contaminations, trained personnel [52].
Nucleic acid amplification test (NAAT) LAMP and PCR detailed in table 1			Sensitive and specific, simple, cheaper, multiplex ability, can detect drug resistant mutations [40].	Restricted to centralised laboratories due to experimental demands, show low sensitivity in smear-negative samples [40].
Micro/nano-fluidics Coupled with existing conventional techniques to meet the demands of POCT for developing regions. Conventional techniques utilise big sized expensive instruments which can be engineered (size minimised) and made cheaper. Their attributes are maintained or enhanced, e.g., a miniaturized flow chamber microfluidic device used to monitor HIV in developing regions [53].			Rapid, simple, label-free, multiplexing potential, economical, sensitive [40], portable, and disposable [53].	Found in centralised laboratory, ongoing research to make them rural-environment friendly [4].

Tuberculosis (TB)

TB is a chronic infection caused by a Mycobacterium tuberculosis (MTB) complex [37] and it is one of the leading causes of death globally for the past 25 years [38] evidenced by the subsequent WHO statistics. Primarily TB affects the lungs while secondary extra-pulmonary TB can develop in the central nervous and circulatory systems and other body organs [1,38]. MTB is airborne and thus get released into the air if an infected person coughs or sneezes. Innate immunity is evoked upon inhalation of the MTB tubercle bacilli into the lungs. Ideally, alveolar macrophages should be able to ingest and destroy the bacilli. However, in most cases, the virulence factor of the MTB exceeds the intrinsic microbicidal capacity of the host phagocytes thus will multiply leading to disruption of the macrophages [39]. In about 2-3 weeks post-infection, T-cell immunity produces antigen specific CD4+ and CD8+ T-cells to activate macrophages to kill the intracellular bacilli. The T-cells also helps in increasing the production of cytokines such as primarily gamma interferon [70] which in turn activates macrophages and stimulates natural killer cells and neutrophils [79,214]. In addition, B lymphocytes produce antibodies against lipoarabinomannan which is a glycolipid and a virulence factor of MTB. Figure 2 illustrates the different stages of the growth of the bacterium from the inhalation stage to development and the release to the atmosphere via sneezing of an infected person. Active pulmonary TB is responsible for the transmission of this disease. At times, the tubercle bacilli can be harboured inside the lungs in a dormant state [1,39]. The Latent infection can progress to active infection if triggered by environmental changes which can weaken the immune system such as pregnancy or presence of other pathogens [1,40]. Hence, diagnosis of the TB bacterium is informed by the pathogenicity of the infection, active or dormant.

In 2011 approximately 1.1 million death cases were reported out of 8.8 million reported TB cases; and WHO estimated a 2 million multi-drug resistant TB outbreak between 2011 and 2015 [40]. In 2014, 9.6 million people were estimated to have contracted the TB bacterium worldwide; 5.4 million men, 3.2 million women and 1.0 million children. Of the 9.6 million cases, 12 % were ascribed to HIV-positive patients. However, out of the 9.6 million estimated cases, only 6 million cases of TB were reported in 2014; 37 % of the cases remained unreported and triggers questions on their management [41]. It is such cases that perpetuate the spread of the air-borne bacterium since the patients are not treated. Hence

effective diagnostic tools are a necessity to close the existing gap between diagnostics and treatment. The 2014 reported cases had higher mortality cases for men 890 000, 480 000 women and 140 000 children. In 2014, multi drug-resistant (MDR-TB) cases were estimated to be 480 000 but only 123 000 cases were reported. In May 2014, WHO embarked on a global end TB plan; by 2030 countries are expected to lower numbers of TB mortality by 90 % and new incidences by 80 % and lighten financial burden of families affected and infected by the epidemic [41]. The pandemic continued and in 2015, 49 million lives were lost due to TB, 6.1 million new cases and 580 000 eligible for MDR-TB treatment [42]. The recent WHO and United Nations SDGs (2016-2035) continue to strive to end the TB endemic. A 90 % reduction in TB mortality and 80 % reduction in new cases is envisioned by 2030. Technological breakthroughs in diagnosis and treatment of TB, eradication of socio and economic consequences of TB, worldwide TB care and prevention are cogent markers which would aid in the attainment of the aforementioned goals [43] (Figure 2).

TB diagnosis

For an active infection, diagnosis is done by chest radiology or sputum collection and microbiological cell culturing [1,44]. Whereas latent infections diagnosis poses a challenge due to absence of know TB symptoms like coughing, sputum production and fever. To mitigate this challenge tuberlin skin test which measures immune response of a person when injected with more than 200 TB antigens is used for latent infections [1,44]. A positive skin test is shown by the patients' response to the antigen via the development of inflammation on the elbow where the antigens are usually injected [1]. Table 2 gives a summary of the advantages and disadvantages of some of the TB diagnostic techniques.

Diagnosis of the TB bacterium proffers challenges due to the slow growth of the bacterium which hampers on-site patient diagnosis [40]. Thus, the lack of symptoms and low bacillary burden during the early stages of infection is a concern. The use of sputum compared to urine or blood complicates the process: i.e., sputum collection, transportation and storage is prone to inappropriate handling and storages which results in poor results [40]. Additionally, the lack of reliable and authenticated TB biomarkers hinders diagnosis. Patients at different stages of infection with different immunization records react differently to treatment. Hence, understanding the pathogen and host reaction during infection is a challenge. An ideal POCT diagnostic assay to mitigate all these afor-enumerated challenges is required [40] (Table 2).

Human Immune Deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS)

HIV is a retrovirus that infects the immune system cells and renders them dead or compromised [54]. The early stages of infection are without physical symptoms. Increase in HIV replication and a rise in the viral load is subsequently accompanied by fatigue, fever, headaches, night sweats and other symptoms 2 weeks after the infection [55]. The immune system thereby responds by producing anti-HIV antibodies a few weeks after the infection and this helps lower the HIV multiplication rate and progressively lowers the viral load. The progression of HIV infection depends on the viral and host immunity factors. For some individuals, it takes about 34 days to have detectable antibodies in the blood whereas some can take up to 3 months [56]. HIV weakens the immune system and the infected person becomes susceptible to multiple common bacterial and viral opportunistic infections such as pneumonia and meningitis and co-infection such as TB, hepatitis, cancer and others [57]. This, in turn, contributes to a more compromised immune system known as AIDS [54]. The first case of HIV was reported in 1981 and it is one of the formidable challenges of our time [58]. Although impressive strides have been taken in the control, prevention and management of HIV and AIDS using antiretroviral therapy (ART) [59] to a point of undetectable viral load, however, it is still a challenge to completely eradicate HIV. The immune cells, macrophages, dendritic cell, T-helper cells or CD4 are knocked down by this globular ribonucleic acid virus which has a very high mutation rate and this mutation rate creates challenges for the development of treatment and diagnosis assays [1]. Figure 3 summarises the HIV life cycle. The HIV parasite fuses with the CD4+ T-helper cells and integrates with the host DNA, replicates, assembly, buds and matures. The matured HIV strains are then released into the blood to attack more CD4+ T-helper cells and further weaken the defence system [60]. The virus can spread through biological fluids (blood, vaginal fluids, semen breast milk) contacts and to counteract the spread of the virus rapid diagnosis and preventative measures are a requirement [1]. The end of the United Nations (UN) MDGs in 2015 recorded a decline in HIV/AIDS new cases and mortality [61]. The UNAIDS/WHO estimated a 35 % (2.1 million) reduction of new cases, ascribed to prevention programmes and ARV treatment [61]. The expansion of the ARV resulted in a 45 % (1.1 million) decline in death cases and by mid-2016 about 18 million people were estimated to be on the ARV treatments [61]. In the MDGs era, ARV extended to middle and low class and their price continued to decrease to make them accessible to everyone and furthermore the SDGs aim to reduce new cases and mortality incidences to less than 500 000 by 2020 [61]. Accurate, sensitive, affordable diagnosis of the virus is one of the key players in achieving the envisioned SDGs.

Standard HIV diagnosis techniques include enzyme immunoassay (EIA) and western blot. EIA is the first generation which detects IgG antibodies and the current third generation which detects IgM antibodies. These techniques are not devoid of challenges as they require trained personnel and twoday visits to the clinic, hence they are not suitable for POCT. Availability of HIV antibodies POCT lateral flows has revolutionized diagnosis with comparable or even better sensitivity (98-100 %) and specificity

(86-100 %) to that of the traditional assays [62]. But early detection is still a challenge, as only after 9 days of infection are the HIV antibodies detectable with the POCT lateral flows [62]. Although the 9 days is better compared to 3-6 weeks western blot turnout time [62]. In the United States of America (USA) in July 2012, an OraQuick test (OraSure technologies) self-administered HIV testing kit was approved by Food and Drug Administration (FDA) and it uses saliva from a mouth swab with a turnout time of 20 - 40 min [63]. The OraQuick test was well received in the USA and even developing countries such as Zambia proven by a published study in 2012 on comparison of the OraQuick test with other two blood-based rapid HIV antibody assays [64]. However, there were also concerns with its sensitivity which was lower on individual users than when it is administered by trained personals [65]. This discrepancy can result in false results and it was not the only challenge, early detection of the virus and false negative results during early infection stages were concerns [65].

However, in a case like South Africa, the use of the OraQuick self-HIV testing kit was received with mixed emotions triggering absurd fears of HIV positive people committing suicide because of lack of counselling [63]. South African pharmacies were burnt from supplying the OraQuick test medical devices even though the medicines and related substance control act no. 161 of 1965 had no direct regulatory system for such [63]. Self-administered kits can allow people to test at the comfort of their homes without feeling judged which can eradicate the issue of late diagnosis [63]. In May 2015, after much deliberation the pharmacy council of South Africa lifted the ban, allowing pharmacies to supply the kits under strict minimum standards [66] (Figure 3).

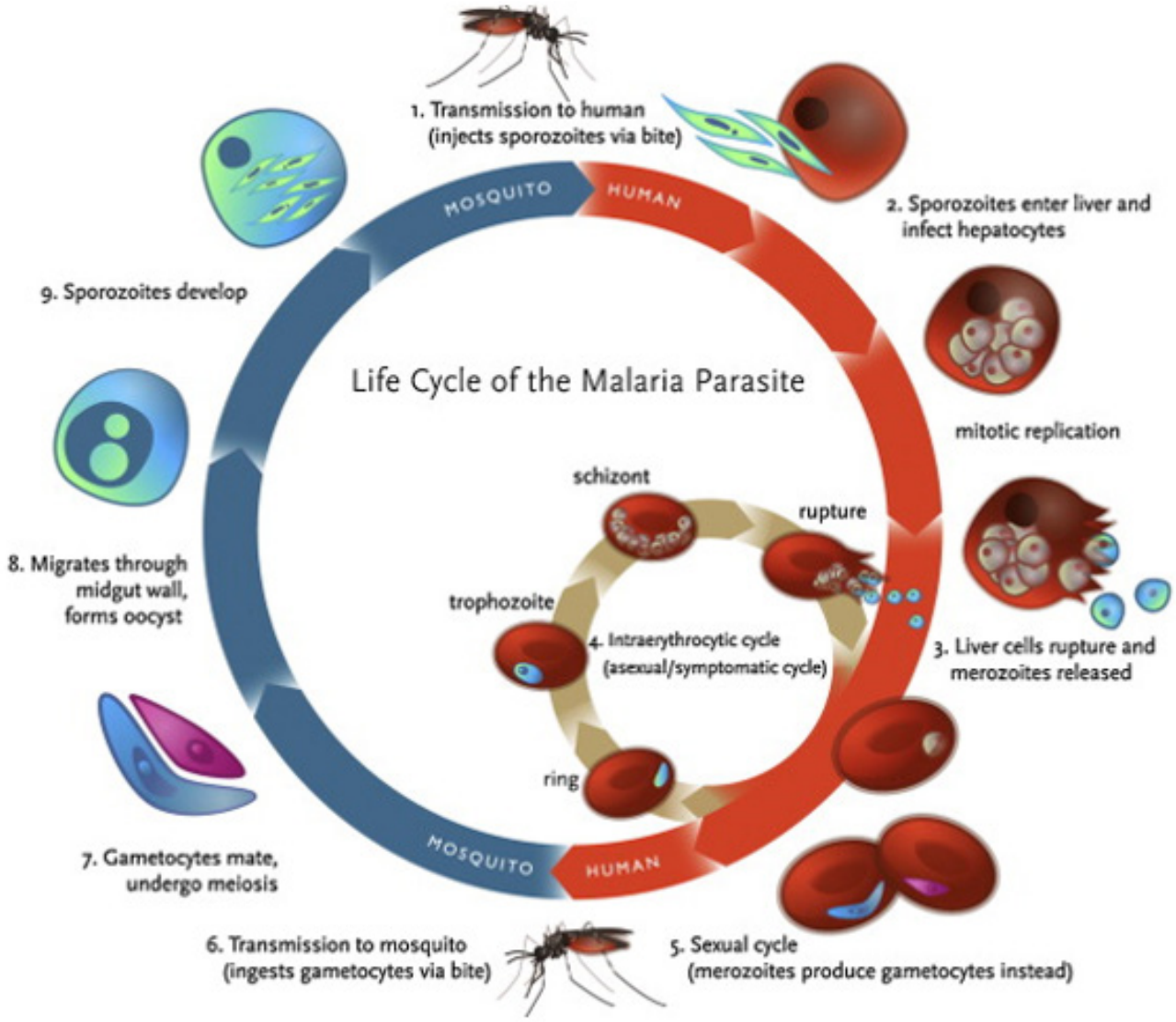

Figure 1: Malaria life cycle. Reprinted from antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread, 41 [17].

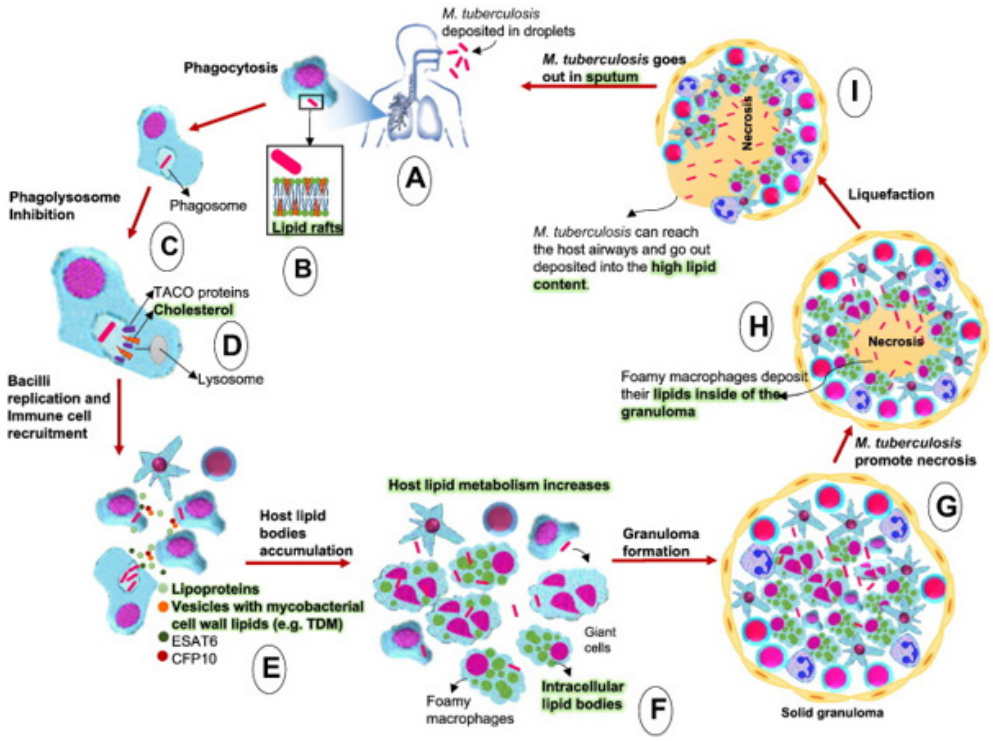

Figure 2: Figure 2: Reprinted from Genetic regulation of Mycobacterium tuberculosis in a lipid-rich environment, 55, Diana [37].

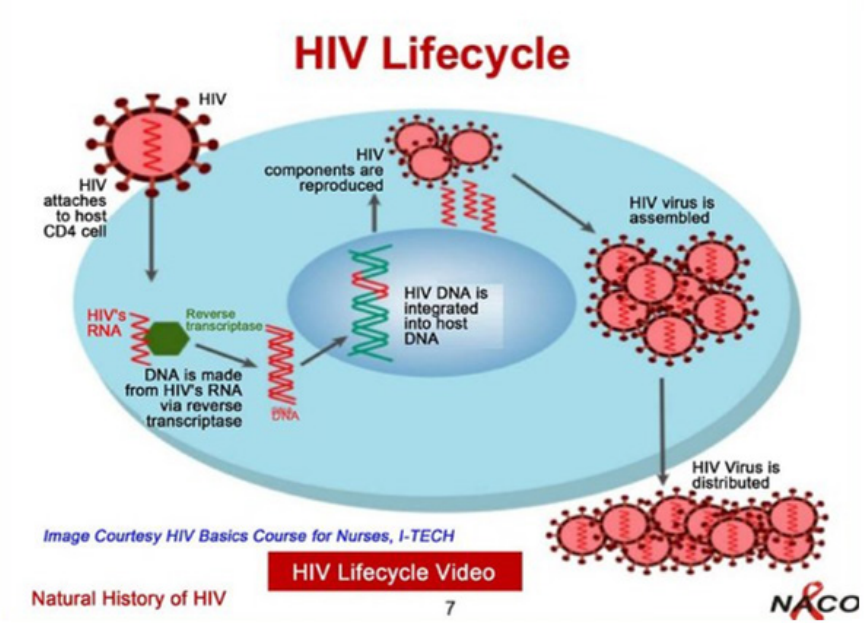

Figure 3: Replication of the HIV virus reprinted by permission from licence CC BY 4.0 [67].

Vibrational Spectroscopy in the Detection Of Infectious Diseases

Optical spectroscopy, i.e., Raman and Fourier transform infrared (FTIR) are fingerprinting tools that can be used in the diagnosis of infectious diseases due to their high sensitivity, specificity in low parasitemia levels, portability, capability and ability to visualise and analyse molecular composition and interactions [68]. The WHO malaria and TB gold diagnosis standards discussed in preceding sections still suffer from low sensitivity and in cases of considerable sensitivity, they are observed to be too complicated and expensive for low-resourced regions. Since the WHO's ambitious goals include health for all, regardless of geographical socio and economical

standards, Raman spectroscopy, although known to be bulky has the potential to be miniaturized into portable sizes and reviewed for diagnosis of infectious diseases. The consideration of the vibrational spectroscopies, Raman and FTIR for detection of infectious diseases such as malaria is not new. Studies have been published confirming their application as detection tools. One of the malaria biomarkers, hemozoin has a distinct fingerprint which can be analysed using the vibrational spectroscopy tools such as Raman and its variants, Surface Enhanced Raman Spectroscopy (SERS), Tip-Enhanced Raman Scattering (TERS) and Resonance Raman spectroscopy (RRS). Figure 4 adapted from wood et al [69] compares the TERS, SERS and RRS on fingerprinting of malaria infected blood cells. The TERS was used to probe the hemozoin crystals from the infected cells vacuole and the AFM images show the hemozoin crystals and characteristic hemozoin Raman peaks were recorded [69]. The imaging of the malaria biomarker, hemozoin is also attained via Raman imaging, a wide field Raman imaging tool using fiber array based spectral translator and tailor made laser illumination system was used to image hemozoin from malaria infected erythrocytes [70]. This approach presented potential for hemozoin analysis in early ring stages of the P. falciparum [70]. Figure 5 details the hemozoin characteristic peaks from the infected cells and images developed using the Raman imaging algorithm. An Attenuated total reflectance-FTIR was coupled with partial least-squares regression models to detect and quantify the malaria parasite at different stages of infection [23] (Figure 4 &5).

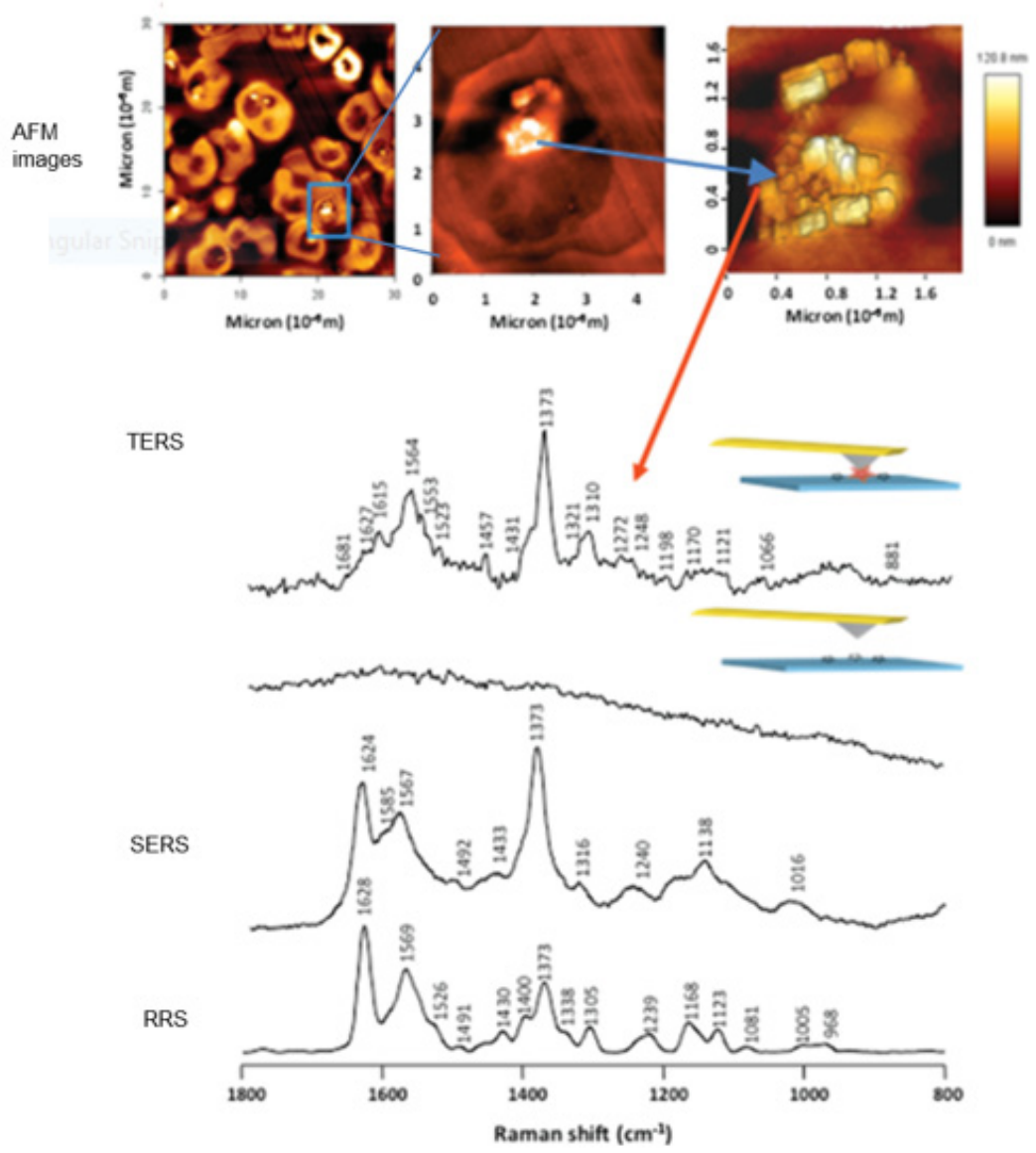

Figure 4: Detection of Hemozoin from infected malaria cells imaged using AFM and molecular fingerprinting using TERS, SERS and RRS. Reprinted (adapted) with permission from [69].

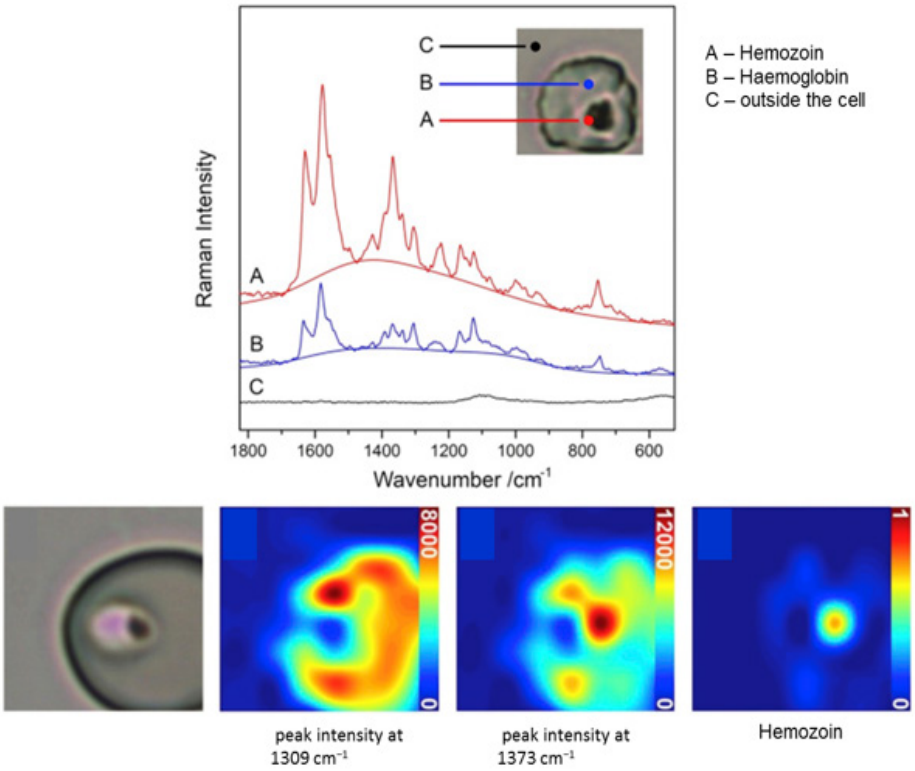

Figure 5: Raman spectra of infected blood cells at different positions and imaging. Reprinted from fiber array based hyperspectral Raman imaging for chemical selective analysis of malaria-infected red blood cells, 894, [70].

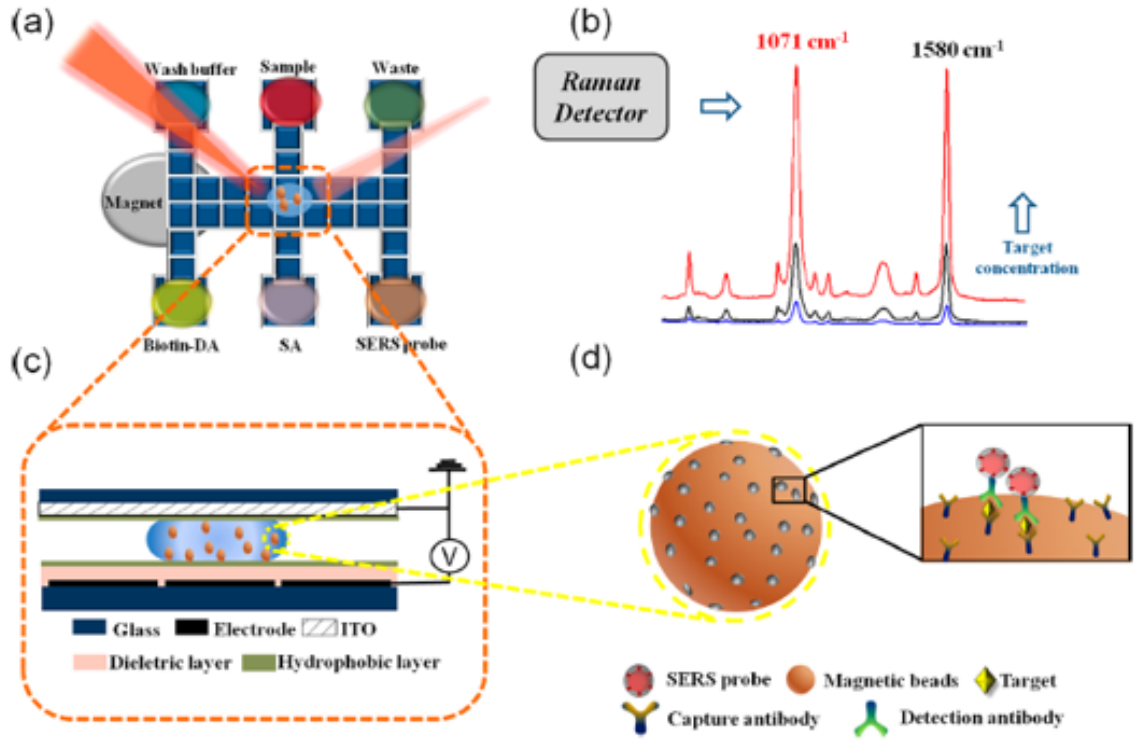

Figure 6: Schematic representation of the DMF-SERS assay (a) DMF-SERS method and bottom plate of DMF chip. (b) SERS peaks for the Raman tag. (c) Side view of the DMF chip coating a droplet of magnetic beads. (d) The sandwich biosensing approach [73].

The application of the vibrational spectroscopy in the detection of biological fluids is growing evidenced by a growing number of peer reviewed proof of concept publications [71]. However translation of the vibrational spectroscopy concepts into real life applications such as detection of infectious diseases in clinics and POCT is still a bottleneck. The vibrational tools present several challenges from the pre-analysis to the analysis stage. Key areas such as sample volume, dilutions, storage, and processing, inter-operator and inter-plate reproducibility need to be standardized to avoid lack of reproducibility. An accurate protocol has to be established via clinical trials [71]. The protocol has to also mitigate the challenges presented by the complexity of the blood i.e., inconsistent data due to variable genetic, ethnicity, age, hormonal differences (gender), and lifestyle (smoking, diet) [71].

Synergising the SERS probes with microfluidics mitigates some of the aforementioned key areas such as sample volume, dilutions, storage, processing, inter-operator and inter-plate reproducibility. Microfluidics detection offers the use of smaller volumes, shorter detection time and this in-turn result in rapidity and reduced cost [72]. The coupling of microfluidics with a powerful fingerprinting tool such as SERS is envisioned to result in fast, highly sensitive and specific diagnostic lab on chip gadgets [72]. Wang et al [73] recently proposed a SERS-based immunoassay with digital microfluidics (DMF-SERS) for rapid, automated and sensitive diagnosis of disease biomarkers. A sandwich approach was used: magnetic beads were conjugated with a capture antibody and used as a solid capture for antigens. A SERS probe was synthesised by labelling detection antibody coated Au core-shell nanostructures with 4-mercaptobenzoic acid. The DMF-SERS immunoassay was tested for quantitative detection of avian influenza virus in buffer and human serum and showed excellent sensitivity and selectivity with less assay time and reduced cost by using low reagent volumes [73]. Figure 6 gives an illustration of the DMF-SERS immunoassay (Figure 6).

Outlook

The world experienced a decline in infectious diseases; especially malaria and HIV and Aids at the end of the United Nations Millennium development goals (MDGs). In the MDGs era, massive strides were taken globally towards better scientific and technological techniques in diagnostics, treatments and prevention of the infectious diseases, i.e., ARV treatments on HIV and Aids patients and malaria and TB medication subsidised by the governments. The MDGs (2000-2015) included compacting diseases, illiteracy, poverty, hunger, environmental deterioration, women abuse or discrimination. It is still a concern that the African region and South East of Asia which are mostly developing regions are still mostly impacted by these infectious diseases, having new and deadly cases recorded at the end of the MDGs era from these regions. Infectious diseases WHO gold standard diagnostic tools discussed in the preceding paragraphs are not resource-constrained region friendly. They are expensive, require trained personnel and at least two visits to the clinic, mainly the sensitive spectroscopy requires a full laboratory with good water supply and electricity. All of these requirements are far-fetched for under-privileged areas which are most impacted by infectious diseases. Hence, the urgent need to produce POCT diagnostic assays which will fit the ASSURED WHO standard. Diagnostic tools should be affordable, sensitive and specific, rapid, require no trained personnel, available to the people and possibly require no use of expensive equipment. Development of these POCT tools is already being explored using nanotechnology, material science and other technologies e.g., development of the self HIV testing kit. The self-testing kit will encourage testing especially in regions where HIV and Aids is still stigmatised. The MDGs achievement is impressing although resource-constrained regions are still burdened by the infectious diseases. Hence the extension of MDGs to 2030's 17 sustainable development goals persisting on achieving healthy lives across all ages. Vibrational spectroscopy (Raman) coupled with lateral flows or microfluidics can be explored for POCT and with the potential for spectroscopy to be miniaturized this could revolutionize diagnostics. However, we cannot ignore that it will come at a cost and hard work to translate the prototypes to functional reproducible POCT gadgets.

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Conflict of Interest

No conflict of interest.

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