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Abstract

Parasitic diseases have a great impact in human and animal health. The gold standard for the diagnosis of the majority of parasitic infections is still conventional microscopy, which presents important limitations in terms of sensitivity and specificity and commonly requires highly trained technicians. More accurate molecular-based diagnostic tools are needed for the implementation of early detection, effective treatments and massive screenings with high-throughput capacities. In this respect, sensitive and affordable devices could greatly impact on sustainable control programmes which exist against parasitic diseases, especially in low income settings.

Proteomics and nanotechnology approaches are valuable tools for sensing pathogens and host alteration signatures within microfluidic detection platforms. These new devices might provide novel solutions to fight parasitic diseases. Newly described specific parasite derived products with immune-modulatory properties have been postulated as the best candidates for the early and accurate detection of parasitic infections as well as for the blockage of parasite development.

This review provides the most recent methodological and technological advances with great potential for bio-sensing parasites in their hosts, showing the newest opportunities offered by modern "-omics" and platforms for parasite detection and control.

Keywords: detection platforms; diagnosis; proteomics; parasitic diseases; sensors; microfluidics.

1. Introduction

Parasitic diseases represent particular challenges for human and animal health mainly in developing countries. They are strongly associated with poverty causing a considerable health and economic impact especially when considering co-introduced and co-invading parasites [1, 2]. Moreover, parasitic infections can be found worldwide and can be potentially introduced from endemic to non-endemic areas mainly due to human and animal population movements and climate change, resulting in their emergence and re-emergence [3, 4]. Neglected parasitic infections are very prevalent especially among children and immunocompromised hosts -even in developed countries-, causing high morbidity and mortality rates [5, 6]. The lack of appropriate diagnostic tools for many of these neglected diseases, combined with their lack of appropriate sensitivity and/or specificity, makes the investigation on new type of detection devices a must.

Zoonotic parasites are also the cause of substantive economic losses in livestock populations [2]. In this sense, the interest in understanding disease transmission among wild and domestic animals, and between them and human population has grown, resulting in the emergence of the "one health approach", which aims to model the transmission of parasitic diseases [7, 8]. Detection and transmission control constitute significant components to the overall management of many pathogen infections (including parasites). Moreover, the rapid diagnosis of many complex parasitic diseases with on time treatments and tailored control measurements is essential to avoid sequelae, comorbidity and economic losses.

Drugs treatment remains as the principal approach for the control of parasites in animals; however parasites have shown molecular resistance mechanisms hampering this strategy. Successful and sustainable control strategies depend on the development of new tools for targeting both parasites/hosts and vectors [9]. To this end, applying new knowledge and techniques in combination with updated mass drug administration programmes is essential.

The Diagnosis of the most health impacting parasites is often cumbersome, where current diagnostic tests for important zoonotic parasitic diseases can provide incorrect results and so lead to unforeseen consequences [10]. Therefore, there is a much-need for rapid, simple, sensitive and affordable diagnostic tests to improve disease control and patient management, mainly in poor-resource settings where

diseases like malaria or human sleeping sickness are endemic. In these scenarios, many people do not have access to laboratory facilities and many barriers technologies, costs and expertise lead to the necessity of incorporate point of care (POC) tests which offer flexibility, reliability and robustness for both, case detection and large population screenings [11].

Outside medical or veterinarian laboratories, rapid on-site diagnosis is very important in order to prevent and manage outbreaks and to apply appropriate prophylaxis, treatment and control programmes [12]. In this sense, biosensor-based tools developed for the diagnosis of pathogens is an emerging issue (131 PubMed papers from 2010 when searching "biosensor diagnosis pathogens" on December 2015) and ultimately a growing area of concern when referring to the diagnosis of parasitic infections (38 PubMed papers from 2010 when searching "biosensor diagnosis parasites" on November 2015).

The main goal of this emerging area of knowledge is to develop devices with multiplex capabilitites as suitable screening methods for the detection of several parasites and their corresponding vectors. Despite the recent advances in bio-sensor technology for infectious and parasitic diseases, they still remain as one of the major causes of mortality and morbidity throughout the world. This review presents current examples and perspectives for integrating modern sensing technologies for the detection of parasitic diseases.

2. Predictive candidates to detect parasitic diseases

Candidate biomarkers for the diagnosis of parasitic diseases must be identified in differentially expressed molecules between healthy subjects and infected patients. The first step towards an effective treatment of parasitic infections is an early detection and later differentiation of the disease progression and/or recurrences over time. Having into account the variations of the individual immune responses to the infection, the identification of candidate biomarkers is a more appropriate approach for reliable and specific parasite identifications than defining a single candidate. Thus, a combination of key hosts and parasite molecules is ideal to be included in target panels with the aim of achieving low overlapping between different diseases and therefore facilitate an integrated diagnosis.

Protein-protein interactions at the host-parasite interplay are highly relevant in the context of modifying protein expression levels and inducing protein expression.

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Identifying these alterations in host protein profiles during infection could facilitate the understanding of disease pathogenesis, host immune response, and identification of potential protein markers for the detection and prognosis of the disease. In this sense, parasitic infections may lead to highly relevant alterations on the level and expression of multiple serum proteins involved in essential physiological pathways (*i.e.* lipid-binding proteins) as well as in the change of very specific host molecules (*i.e.*- the host erythrocyte membrane proteins during malaria infection) [13, 14].

Investigating the metabolic consequences of parasitic infections in the host is now feasible based on modern technological advances. Experimental infections with single or multiple parasite species may allow the discovery of specific or common biomarkers, although they should be further validated in free living populations [15]. To this end, for example it has been shown by serum proteomics of *Eimeria sp* infected chickens that proteins usually not detected in the blood like those associated with mitochondrial metabolism are good candidates for time-course studies in coccidiosis. However, the host genetic background leading to different levels of susceptibility is a key factor in the alteration profile of proteins [16].

Regarding parasite derived molecules, the strategy to detect circulating antigens has the potential to discriminate active from past infections. Assessment of antigens during a given infection and specifically the excreted-secreted or surface-exposed parasite proteins, should be the best for an accurate immunodiagnostic procedure. The study of the surfome in parasites is revealing novel molecular targets for specific diagnosis. Many of these molecules are represented by glycoproteins located at the extracellular region attached to the plasma membrane, although antigenic variability at this level makes the selection of molecules cumbersome [17, 18]. This highlights the importance of comparative surfome analysis to increase the chance to find specific targets in parasites, leading to differential surface markers useful to avoid potential misdiagnosis like for example of Chagas' disease [17]. In addition, the potential detection of fine post-translational modifications creating neo-epitopes during specific parasitic disease pathogenesis could lead to monitor disease activity, like in many human diseases [19].

Epitope mapping studies aimed to identify unique diagnostic molecules from polymorphic immune-dominant antigens in parasites using computational methods is also a promising area [20]. For instance, the identification of many novel epitopes with diagnostic potential has been proved for the protozoan *Trypanosoma cruzi* [21].

Moreover, the detection of new diagnostic epitopes from circulating antigens is also a practical diagnostic strategy.

Some of the putative new protein biomarkers to detect parasites are likely to be present in biological fluids at extremely low concentrations and protected inside secreted microvesicles. Microvesicle-based secretion seems to be a general mechanism for protein secretion by protozoan parasites. It is well reported that the biomolecular cargo (*i.e.* proteins, lipids, nucleic acids) inside these microvesicles is involved in signalling for parasite infection and its survival therefore it could be also exploited in disease treatments [22, 23]. Since extracellular vesicles are highly immunogenic, they can be considered as suitable candidates to detect parasitic diseases [23].

3. Proteomic platforms for the identification of parasite biomarkers.

Parasites have complex life-cycles and redundancy molecules can be found in many infectious processes, posing additional difficulties for their specific identification by classical biochemical approaches. Additionally, parasitic infections may alter the metabolic activities in their host being these alterations the basis of metabolic fingerprint approaches for understanding the metabolic consequences of the infections. Therefore, these could be considered as a source for novel diagnostic or prognostic biomarkers [15]. Nowadays, modern -omic technologies are offering high-throughput strategies for such a difficult system biology exploration, with the essential support of novel bioinformatic tools [24, 25].

The identification of biomarkers of parasitic infections by differential protein profiling of a specific host or parasitic molecules is a promising area; mainly based on induced changes by parasites at post-translational modifications, peptide degradations or protein variants [26]. Tissues (human and animal) and proximal bio-fluids could contain molecular information on the physiological and pathological state of the organism, since these tissues and bio-fluids are the route and destination of parasites and/or their secretions. The proteomic characterisation of proximal bio-fluids may provide useful and comprehensive information for diagnostic, prognostic and predictive biomarkers. The challenge is the detection of very low abundant protein circulating biomarkers -these kind of molecules provide a greater amount of downstream information content than nucleic acids- that may be essential for early diagnosis [27].

3.1 The Potential of Mass Spectrometry

Briefly, Mass Spectrometry (MS) is a robust, versatile and sensitive analytical technology allowing high-throughput detection with mass accuracy, precise quantisation and verification of protein variants, splice isoforms, metabolites and disease-specific post-translational modifications from tissues, body fluids or cell cultures [28, 29]. Highly sensitive technologies and high-throughput systems -like MS- are promising tools for novel biomarkers since MS allows characterisation of the fine tune changes in proteins, -including differences at population level- [30].

Commonly, MS-based proteomics approaches are a suitable resource to identify biomarkers for the detection of parasites as the main technique or coupled with other proteomic and conventional biochemical techniques. For instance, this is the basic for the identification of potential malarial markers in patient´s sera and recently applied to identify specie-specific proteins in infected patients [31, 32].

De Bock and colleagues highlighted the potential of MS-based technologies (*i.e.* surface-enhanced laser desorption/ionisation time of flight mass spectrometry, SELDI-TOF-MS) as research tools to interrogate protein-based biomarkers in parasitic diseases. In general, SELDI-TOF determines differential specific protein profiles (in particular, low molecular weight molecules) in proximal bio-fluids. The above mentioned technology has also been applied in parasitic diseases by sensing unusually truncated host proteins in response to *Trypanosoma cruzi* infection with high sensitivity and specificity [33]. Moreover, matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) is a valuable diagnostic technique already applied for detecting and differentiating *Entamoeba Spp* or *Babesia canis canis* infections, and becoming a miniaturised bioanalytical tool for detecting and discerning proteins from bio-complex samples [34-36]. MALDI mass spectrometry has applications in biomarker discovery, pathogen identification and has great potential for lipid-based biomarker sensing [37, 38]. For the identification of vectors like mosquitoes, MALDI-TOF allows accurate identifications which are important to elucidate their role as vectors. Importantly, the technique has also the potential for rapid in one-shot dual identifications (vectors and pathogens) [39, 40]. The utility of MS in routine analyses to point out markers of parasitic infections in bio-fluids and tissues was proved to be effective in many recent publications (Table 1), but still needs to be broadly used for research and clinical diagnosis. Many pre-analytical protocols during parasite sample preparation must focus on maximising the number of protein identifications on perform highly-reproducible assays.

Other MS-based techniques, not predominately applied in diagnostic parasitology, but that hold great potential for the development of this area are:

(i) MS imaging is an analytical tool providing information on the spatial distribution and relative abundance of biomolecules in tissues [37]. Its power depends on an unbiased preliminary knowledge of molecular identities required, its ability to distinguish between diseases with similar histological characteristics, the different type of molecules detected, the quality of tissue preservation/conservation and the unique applications for clinical diagnostics [60]. It would probably be advantageous to correlate molecules and pathogenesis of impacting diseases like severe malaria, severe hepatosplenic schistosomiasis and cutaneous forms of leishmaniasis.

(ii) An emerging technology for pathogen detection is PCR–electrospray ionisation mass spectrometry, a versatile technique to characterise multiple strains and organisms having high potential to identify tick-borne pathogens [61, 62].

(iii) Multiple reaction monitoring mass spectrometric assays can be used in order to quantify multiple protein isoforms, specific proteins or modified peptides [63]. This technique requires enriched samples to accurately analyse modified biomolecules like lipopolysaccharides, glyco-sphingolipids and glycoproteins, which are highly relevant antigens of parasites [64].

(iv) Direct identification of compounds originated from pathogens physiology/metabolism within hosts can be done by metabolomic approaches [65]. Metabolomics profiles based on MS strategies detecting variations in differential metabolic signatures in cells, tissues or body fluids, may open a way for sensing parasites. When trying to detect and understand host or parasite derived metabolites at low concentration, capillary electrophoresis mass spectrometry with electrospray ionisation and gas-chromatography mass spectrometry offer valuable discriminatory power [66-68]. It could be also applied to time-course and accumulation of drug metabolites after treatment of parasitic diseases.

3.2 Nanoproteomics

Nanoproteomics is a new ''-omic'' emerging discipline coming from the advances and integration of nanotechnologies and proteomics, useful for rapid diagnostic screening at nanometer scale in the clinical practice. Nanotechnology has the potential to minimise most of the problems related to the proteomic technologies (*i.e* complexity, protein modifications and allows new methodological approaches such as

multiplexing thus opening new biologically relevant insights) providing advantages at the detection level [69].

Multiplexing is an important feature that may facilitate the simultaneous study of different parameters within a unique platform. Protein and glycan microarrays, bead arrays or 3D printings are some of the methodologies included in this field within on-demand diagnostics platforms. The use of dynamic sensing arrays based on micron and/or nano-sized beads allows increasing the level of multiplexing with the potential to be adaptable in microfluidic devices for immunoassays [70]. Protein microarrays are miniaturised immunoassays in an array format which allows the study of thousands of proteins simultaneously, offering the opportunity to perform high-throughput screening for new biomarkers of infectious and parasitic diseases at global level [71, 72]. Moreover, peptide arrays could be powerful tools for deciphering epitope localisation: a combination of protein arrays followed by epitope scanning. Peptide chips could be a good strategy for the identification of potential immunodominant antigens and for epitopes description [73]. Biochips immuno-like polymer membranes for POC sensing of proteins in serum samples is also an interesting field [74]. In fact, novel single-domain antibodies (*i.e.* scFvs, nanobodies), may offer interesting advantages over monoclonal antibodies if applied to these methodologies [75, 76].

Glycan microarrays are also powerful tools for biomarker discovery. These microarrays have the potential to identify carbohydrate antigens and improve the serodiagnosis of different parasitic infections, although advanced methods for the synthesis, isolation, and characterisation of glycans are still required [77-79]. The diagnostic potential of glycans found in Leishmania, Schistosoma and Trichinella parasites points outs their relevance.

3.3 Subcellular proteomics

The analysis of the composition of particular purified cellular compartments is gaining importance in diagnostic research. Extracellular vesicles represent a promising source of circulating biomarker cargo encapsulated in a lipid coat. Among them, exosomes, 40-100 nm in size vesicles, which are important in inter-cellular communications, play critical roles in many biological functions. Exosome surface

protein contents can be a rich source of biomarkers in blood samples and proximal fluids. These vesicles could have a high potential as optimal diagnostic targets based on the differential display of specific exosomal protein markers. Exosome-MS protein libraries prepared by either the shave -for surface protein exosomal markers- or complete exosome material, produce ideal peptide libraries for MS/MS analysis.

There is accumulating evidences of the release of inter-communicating extracellular vesicles in parasitic diseases, being an integral part of the parasite's infectious life cycle [80, 81]. The potential that proteomics, especially mass spectrometry, show for sensing and deciphering the cargo of these parasite or host secreted biomarker enriched microvesicles is very high in the field of parasitic diseases diagnosis [82]. The detection of parasitic derived microvesicles would be highly valuable for example to diagnose and guide management of chronic and complex asymptomatic diseases, such as cystic echinococcosis or in vector transmitted infections like Leishmaniasis, in which exosomes are new identified virulence factors [81].

Molecules in the exosome cargo like the microRNAs have also great potential for the diagnosis of parasitic diseases [83]. Other type of analytes that could be studied in the exosome space, *e.g.* lipids, might represent good biomarkers to investigate in the near future. All these provide an important base to continue researching in parasite derived exosomes as diagnostic targets and demonstrating their utility as clinical biomarkers.

4. Parasite testing: biosensor based platforms

There is an important demand in parasitic disease diagnostics for portable and highly sensitive systems. Novel detection platforms have the potential to develop robust, multiplexed ultrasensitive protein detection devices with high efficiency, high data quality, and cost-effectiveness for the identification of pathogens and disease biomarkers in both well-equipped and/or limited clinical facilities [84].

Biosensors are also new molecular technologies that attempt to overcome many of the detection limits due to the low abundance of key biomarkers [85]. Some of these technologies have impressively improved sensitivity compared to conventional immunoassay approaches [86]. However, few biosensors have been developed and commercialised for the detection of infectious or no infectious diseases, but none of them for parasitic diseases.

Biosensors can be used as point-of-care devices to detect host and parasite virulence and specific biomarkers, being good alternatives to current standard methods [87]. These biomarkers can be transferred to a biosensor format for multiplexed ultrasensitive sensing directly from proximal body fluids, enabling simultaneous detection of specific, to several evolutive forms, antibodies. This biomarker serves as revolutionary POC diagnostic technology to improve the prevention, control and treatment of parasitic diseases. The inclusion of species-specific markers for differential diagnosis and polymorphism detections would directly impact sensing and help to understand parasites. The integration of high-throughput -omic data, which is relevant to essential host-parasite interaction networks, within these biomarkers appears as the most accurate scenario to understand and guarantee infection status.

These devices have the potential to identify single molecules without the use of microscopes and moreover the same device could potentially be used for several different tests for biomarkers and bio-particles, providing an interesting alternative to standard tests like PCR [87]. This could be very advantageous in low-resource settings where people may not be well trained even in standard test and few technologies are well-established at a POC level, or where the conditions are not appropriated for analysis. These devices will be particularly useful to detect invasive infections or to prevent further disease spread, especially in populations at extreme poverty that still rely in conventional parasitological techniques.

Advanced developments for onsite diagnosis could come from single cell proteomic studies with a microfluidic antibody capture chip platform, through the use of capture antibodies which would be able to detect target markers in real clinical practice [88]. However, the impact of microfluidics on interesting markers like for example exosomes is still small. Therefore, interfacing biosensors with mass spectrometry is leading to the high-resolution identification of macromolecules and thus search for parasitic targets [89]. The interrogation of protein biomarkers in specific cell types and during defined periods in hosts constitutes a potential emerging area of research to provide key advances in the field of parasitology. This could be especially relevant for understanding (i) anti-parasite immune responses in different clinical forms of parasitic diseases (*i.e* Chagas disease) and their role driving the development of this disease and (ii) the systemic impact of parasitic infections.

The development of specific methodologies is still required to accomplish and integrate all the above technologies in a high quality and robust sensing device. The

ultimate advances in bio-fabrication techniques allow creating biosensors with living cells in 3D to more closely model the in vivo cell environment, having these devices the potential to combine bio-sensing and therapeutic treatments [90]. The implementation of these tests on clinical applications might positively influence animal and human clinical management as well as significantly reduce costs.

4.1 The potential of biosensors to detect and diagnose parasites

The diagnosis of many parasitic diseases relies on showing parasites in tissue samples and on standard tests, nevertheless there are still gaps in the diagnosis of well know parasites or even in distinguishing between species and subspecies [91] Table 2). The lack of diagnostic tests influences decisively health care decisions. In general, parasitological techniques oriented to detection by microscopic examination have many disadvantages such as: they are mainly invasive, leads to misdiagnosis, require expert microscopists, they are time consuming and they possess lack of accuracy [112]. Moreover, many rapid diagnostic tests lack accuracy, validation, or both. At hospital level, but even more in the field, there is an increasing demand for better diagnostic tests to detect parasitic diseases at early stage.

The pathogenicity and life cycles of parasites are highly complex and governed by the parasite-host interactions. The trend is, instead of testing for a single biomarker, to identify panel of biomarkers that together may be better predictors of clinically relevant parasites towards efficient decision-making in individual POC settings. As commented previously, MS and proteomic techniques can assist as standard tools for the identification of infection induced up and down regulations that may serve as specific protein biomarkers. Moreover, host-derived metabolic and specific pathogenicity sensors would also play a role modulating parasitic disease progression to ensure survival and long-term persistence [113, 114].

In this sense, the infection and persistence by protozoan parasites is associated with changes in host tissue protein composition, highlighting that both parasite and host-derived molecules modulate disease progression [113, 115]. For instance, these kind of bidirectional protein signatures have been identified by proteomics in *Plasmodium falciparum* [116]. Also, in the management of the complex Echinococcosis, was recently suggested that a combination of markers would be highly desirable for the follow-up of threated patients to avoid recurrence [110]. Moreover, an

integrative approach that looks for an effective diagnosis was also suggested for Schistosomiasis [117].

4.2 The critical role of biosensors in important parasitic diseases.

The broad technologies underlying biosensors have experienced many developments, with the goal to enable small, sensitive and easy-to-use devices. As mentioned in previous points, micro- and nanotechnologies can offer new technological tools and potential applications for developing bio-sensing devices for infection´s biomarkers. Many of these biosensors have proven to be useful for the detection of pathogen signatures and circulating proteins in patients [118]. The access to timely and accurate diagnostic tests has a significant impact in the management of many parasitic diseases like malaria.

At present, many microfluidic platforms are under development in order to address the main disadvantages presented above. Microfluidics provide an ideal interface for the manipulation of cells (*i.e.* red blood cells) or even the microorganisms in a complete integrated system that can be fabricated in mass-production at low costs (table 3). These devices have the potential of contributing to the diagnosis, control and treatment of malaria. In the near future, by using a drop of blood obtained from a finger pick, these assays could be automated to reduce human interventions in sample analysis for easy and massive large population screenings.

Sissel Juul and colleagues developed an impressive device, based on droplet microfluidics, in 2012 [137]. The microfluidic device is able to specifically and sensitively detect malaria-causing *Plasmodium* parasites employing isothermal conversion of single DNA cleavage-ligation events catalysed specifically by the *Plasmodium* enzyme topoisomerase I and detectable at the single-molecule level. This device allows for sensitive, specific, and quantitative detection of all human-malaria-caused by *Plasmodium* species in single drops from whole blood with a detection limit of less than one parasite/μL.

Very recently Warkiani and colleagues [138] developed a highly integrated system that allows enrichment and purification of malaria parasites from whole blood using a label-free, shear-modulated inertial microfluidic device. From 2 to 10 parasites were separated per millilitre and quantified using qPCR. This technique is approximately 100-fold more sensitive than conventional microscopy analysis of thick

blood smears and ideal for further integration into an automatic system with downstream detection for POC diagnostic devices.

Red blood cells (RBCs) infected with malaria can be easily detected using microfluidic devices by looking at the morphological changes on the surface of the RBCs. Using controlled surface roughness and shear forces in a microfluidic channel malaria infected RBCs can be slowed and eventually immobilised on the roughened surface from whole blood. Although not well optimised for being used as POC device, it is ambitioned to be an alternative biomarker for malaria diagnosis [124]. Following the same concept of looking at RBCs, Quan Guo and coworkers [128] developed a microfluidic technique for measuring the deformability of single RBCs based on their ability to deform through micrometre-scale constrictions. Although slow and tedious to extract information from the device is able to distinguish among uninfected RBCs and RBCs with various stages of *P. falciparum* infection.

Other ways of parasite detection are the fabrication of special flow-through separator structures inside the microfluidic channel for trapping parasites [139] or the apertures designed by Chunxiao Hu and collaborators [140] that form a trap were the parasite are trapped around a mid-point of its body. This type of traps have been used for differentiate plant parasitic nematodes by their stylet activity trough integrated electrodes that record electrical signals.

An interesting review reported some relevant biosensors developed in POC system for an important vector borne infection as dengue, highlighting their enormous potential in this field [141]. In general, technologies based on biosensors have been applied in diagnostic investigations of a wide range of parasites as can be seen in Table 4. In this way, advanced research through the integration of different techniques for multiplexing and high-throughput analysis on a chip might lead to the development of multi-parasite detection devices highly advantageous for tropical parasitic diseases [161, 162]. Moreover, microfluidic devices open new avenues to investigate full parasite behaviour [163, 164] and parasite drug response [165] in order to design new strategies to fight them. These technologies are also applicable in toxicology and drug discovery programmes for human metabolic studies and degenerative diseases.

In particular rapid developments are occurring in the field of paper based analytical devices (μPADs). μPADs are a new type of analytical platforms for ASSURED diagnostic tests (Affordable, Sensitive, Specific, User friendly, Rapid and robust, Equipment free, Deliverable to end users, *World Health Organisation*). These

devices should be simple to use, portable, inexpensive able to detect multiple analytes, and usable in use small volumes of sample. Therefore the perfect tools for parasite detection in developing countries are coming. For instance, Horning and co-workers [166] developed a paper microfluidic cartridge for the automated staining of malaria parasites with acridine orange prior to microscopy. The cartridge enables simultaneous, sub-minute generation of both thin and thick smears of acridine orange stained parasites, and has the potential to be used in limited-resources settings (Figure 1).

In the tropics and in the resource-limited settings, water-borne parasites are among the most important parasitic diseases and many obstacles prevent their detection [167]. There is a need for rapid and simple screening of water sources to protect human health from water-related diseases. Currently, information about compact imaging systems incorporating a chip-scale microscope brings light to the diagnosis of major enteric parasites to save many lives [168]. Also, devices can track infectivity as early as 12 h post-infection, faster than other state of the art techniques [169]. Therefore, efforts are needed to facilitate market entry of these new technologies and facilitate mechanisms for their implementation. It is believed that PADs represent a realistic alternative for low cost, mass production and marketable devices. Moreover, the transition of paper based microfluidic devices from the laboratory into the market need to be accomplished by providing the effective fluid flow control on paper and developing paper compatible easy and cheap sensing mechanisms.

Other important parasites are those food-transmitted, which are generally under-detected. These food-borne parasites have complex life cycles, which made difficult their control. In addition, few food-borne parasitic pathogens are effectively monitored in food [170]. Actually, there is a tremendous need to track back food-borne infections using new technologies such as biosensors due to their high specificity and potential to decrease the detection times [171].

5. Conclusions and Perspectives

An efficient diagnosis is very important for the prevention and treatment of infectious and parasitic diseases. Bio-sensing devices based on a high-throughput format could have a favourable impact on disease screening and control implementation. New proteomics developments and their adaptation to POC may allow in a near future to produce low cost devices, increasing the sensitivity and shortening the time of detection compared to conventional tools.

In this sense, there is an important need to start developing and commercialising devices focusing on POC diagnostics applications in human and veterinary parasitology. These devices would favour the overall health of people in developing countries by taking broad and on time prevention and control measures and by assessing the treatment efficacy and facilitating the understanding the distribution patterns of hosts and vectors of important zoonotic parasitic diseases. Moreover, key biosensors might allow the on-site molecular characterisation of multi-parasite species bringing important information on the complex host-parasite interplay.

The convergence of proteomics and nanotechnology more likely will provide specific immune-sensors and immune-assays for detecting biomarkers related to infections. In this regards, the development of portable POC diagnostic tools for detecting circulating exosomes as biomarkers, therapeutic targets and signalling molecules of parasitic origin is a feasible and important goal in parasitology research. The integration of data from detected parasitic molecules and changes detected in host immune and metabolic responses to the infection might provide detailed sensory information for precise molecular-level diagnostics and monitoring tools.

Acknowledgements

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Figure legends:

Figure 1: New type of analytical paper microfluidics for sensing parasites. A) A 3D representation of the cartridge. B) A top-down view of the cartridge. C) A cross-section of the cartridge, emphasizing the slanted nature of the coverslip during use, which permits imaging in both thick (many cells) and thin (single cell) regions. Reproduced with permission from [Matthew P. Horning et al 2014] © 2014, The Royal Society of Chemistry.