

Sensing parasites: proteomic and advanced bio-detection alternatives

Carlos Sánchez-Ovejero¹, Fernando Benito-Lopez², Paula Díez^{3,4}, Adriano Casulli⁵,
Mar Siles-Lucas¹, Manuel Fuentes^{3,4*}, Raúl Manzano-Román^{1*}

¹ Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC), 37008 Salamanca, Spain.

² Analytical Chemistry Department, Universidad del País Vasco UPV/EHU, 01006 Vitoria-Gasteiz, Spain.

³ Department of Medicine and General Cytometry Service-Nucleus, Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), 37007 Salamanca, Spain.

⁴ Proteomics Unit. Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), 37007 Salamanca, Spain.

⁵ Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità. Viale Regina Elena 299 - 00161 Rome, Italy

***Corresponding authors:**

- Manuel Fuentes, PhD. Department of Medicine and General Cytometry Service-Nucleus, Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL). Avda. Universidad de Coimbra S/N, 37007 Salamanca, Spain. Tel.: +34 923294811; Fax: +34 923294743.

E-Mail: mfuentes@usal.es.

- Raúl Manzano-Román, PhD. Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC). Cordel de Merinas 40-52. 37008 Salamanca, Spain. Tel.: +34 923219606; Fax: +34 923219609. E-Mail: raul.manzano@irnasa.csic.es.

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.

Drug 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 December 2015).

The main goal of this emerging area of knowledge is to develop devices with multiplex capabilities 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.

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 specific hosts 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 -this kind of molecules provides 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 quantitation 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 basis for the identification of potential malaria 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-MS, 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-MS 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 MS, 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 metabolomics 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 MS with electrospray ionisation and gas-chromatography MS 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) providing advantages at the detection level and

allowing new methodological approaches such as multiplexing thus opening new biologically relevant insights [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 the global level [71, 72]. 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 out 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 are 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 MS, shows 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 newly identified virulence factors [81].

Molecules in the exosome cargo like the microRNAs have also great potential for the diagnosis of parasitic diseases [83]. Another 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 POC 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 in populations 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, 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 MS 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 lacking of accuracy [112]. Moreover, many rapid diagnostic tests lack accuracy, validation, or both. At the hospital level, but even more in the field, there is an increasing demand for better diagnostic tests to detect parasitic diseases at an 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 the 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 provides an ideal interface for the manipulation of cells (*i.e.* red blood cells) or even the microorganisms in a completely 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 envisioned 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 where the parasite is trapped around a mid-point of its body. These types of traps have been used for differentiating plant parasitic nematodes by their stylet activity through 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 (μ PADs). μ PADs 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 using 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 characterization 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

This research was funded by HERACLES project (<http://www.heracles-fp7.eu/>) under the Seventh Framework Programme of the European Commission. FBL acknowledges the support of the Ramón y Cajal programme (Ministerio de Economía y Competitividad). We also gratefully acknowledge financial support from the Carlos III Health Institute of Spain (ISCIII, FIS PI14/01538 and FIS PI12/00624), Fondos FEDER (EU) and Junta Castilla-León BIO/SA07/15. The Proteomics Unit belongs to ProteoRed, PRB2-ISCIII, supported by grant PT13/0001. P.D. is supported by a JCYL-EDU/346/2013 Ph.D. scholarship.

References

- [1] Lymbery AJ, Morine M, Kanani HG, Beatty SJ, Morgan DL. Co-invaders: The effects of alien parasites on native hosts. *International journal for parasitology Parasites and wildlife*. 2014;3:171-7.
- [2] Torgerson PR. One world health: socioeconomic burden and parasitic disease control priorities. *Veterinary parasitology*. 2013;195:223-32.
- [3] Lipkin WI. The changing face of pathogen discovery and surveillance. *Nature reviews Microbiology*. 2013;11:133-41.
- [4] Carlier Y, Truyens C, Deloron P, Peyron F. Congenital parasitic infections: a review. *Acta tropica*. 2012;121:55-70.
- [5] Yansouni CP, Merckx J, Libman MD, Ndao M. Recent advances in clinical parasitology diagnostics. *Current infectious disease reports*. 2014;16:434.
- [6] Barry MA, Weatherhead JE, Hotez PJ, Woc-Colburn L. Childhood parasitic infections endemic to the United States. *Pediatric clinics of North America*. 2013;60:471-85.

- [7] Godfrey SS. Networks and the ecology of parasite transmission: A framework for wildlife parasitology. *International journal for parasitology Parasites and wildlife*. 2013;2:235-45.
- [8] Patterson JE, Ruckstuhl KE. Parasite infection and host group size: a meta-analytical review. *Parasitology*. 2013;140:803-13.
- [9] Lustigman S, Geldhof P, Grant WN, Osei-Atweneboana MY, Sripa B, Basanez MG. A research agenda for helminth diseases of humans: basic research and enabling technologies to support control and elimination of helminthiasis. *PLoS neglected tropical diseases*. 2012;6:e1445.
- [10] Schwarz NG, Loderstaedt U, Hahn A, Hinz R, Zautner AE, Eibach D, et al. Microbiological laboratory diagnostics of neglected zoonotic diseases (NZDs). *Acta tropica*. 2015; doi: 10.1016/j.actatropica.2015.09.003.
- [11] Peeling R, Mabey D. Point-of-care tests for diagnosing infections in the developing world. *Clinical Microbiology and Infection*. 2010;16:1062-9.
- [12] Mohd Hanafiah K, Garcia M, Anderson D. Point-of-care testing and the control of infectious diseases. *Biomarkers in medicine*. 2013;7:333-47.
- [13] Khoontawad J, Laothong U, Roytrakul S, Pinlaor P, Mulvenna J, Wongkham C, et al. Proteomic identification of plasma protein tyrosine phosphatase alpha and fibronectin associated with liver fluke, *Opisthorchis viverrini*, infection. *PloS one*. 2012;7:e45460.
- [14] Gonzalez-Diaz H, Munteanu CR, Postelnicu L, Prado-Prado F, Gestal M, Pazos A. LIBP-Pred: web server for lipid binding proteins using structural network parameters; PDB mining of human cancer biomarkers and drug targets in parasites and bacteria. *Molecular bioSystems*. 2012;8:851-62.
- [15] Balog CI, Meissner A, Goraler S, Bladergroen MR, Vennervald BJ, Mayboroda OA, et al. Metabonomic investigation of human *Schistosoma mansoni* infection. *Molecular bioSystems*. 2011;7:1473-80.
- [16] Gilbert ER, Cox CM, Williams PM, McElroy AP, Dalloul RA, Ray WK, et al. *Eimeria* species and genetic background influence the serum protein profile of broilers with coccidiosis. *PloS one*. 2011;6:e14636.
- [17] Wagner G, Eiko Yamanaka L, Moura H, Denardin Luckemeyer D, Schindwein AD, Hermes Stoco P, et al. The *Trypanosoma rangeli* trypomastigote surfaceome reveals novel proteins and targets for specific diagnosis. *Journal of proteomics*. 2013;82:52-63.
- [18] Hull R, Dlamini Z. The role played by alternative splicing in antigenic variability in human endo-parasites. *Parasites & vectors*. 2014;7:53.
- [19] Karsdal MA, Henriksen K, Leeming DJ, Woodworth T, Vassiliadis E, Bay-Jensen AC. Novel combinations of Post-Translational Modification (PTM) neo-epitopes provide tissue-specific biochemical markers--are they the cause or the consequence of the disease? *Clinical biochemistry*. 2010;43:793-804.
- [20] Lian Y, Ge M, Pan XM. EPMLR: sequence-based linear B-cell epitope prediction method using multiple linear regression. *BMC bioinformatics*. 2014;15:414.

- [21] Carmona SJ, Sartor PA, Leguizamon MS, Campetella OE, Agüero F. Diagnostic peptide discovery: prioritization of pathogen diagnostic markers using multiple features. *PloS one*. 2012;7:e50748.
- [22] Britton C, Winter AD, Marks ND, Gu H, McNeilly TN, Gillan V, et al. Application of small RNA technology for improved control of parasitic helminths. *Veterinary parasitology*. 2015;212:47-53.
- [23] Mantel PY, Marti M. The role of extracellular vesicles in Plasmodium and other protozoan parasites. *Cellular microbiology*. 2014;16:344-54.
- [24] Cantacessi C, Campbell BE, Jex AR, Young ND, Hall RS, Ranganathan S, et al. Bioinformatics meets parasitology. *Parasite immunology*. 2012;34:265-75.
- [25] Cantacessi C, Hofmann A, Campbell BE, Gasser RB. Impact of next-generation technologies on exploring socioeconomically important parasites and developing new interventions. *Methods in molecular biology (Clifton, NJ)*. 2015;1247:437-74.
- [26] De Bock M, de Seny D, Meuwis MA, Chapelle JP, Louis E, Malaise M, et al. Challenges for biomarker discovery in body fluids using SELDI-TOF-MS. *Journal of biomedicine & biotechnology*. 2010;2010:906082.
- [27] Qoronfle M, Lindpaintner K. Protein biomarker immunoassays opportunities and challenges. *Drug Discovery World*. 2010;11:19-28.
- [28] Baker ES, Liu T, Petyuk VA, Burnum-Johnson KE, Ibrahim YM, Anderson GA, et al. Mass spectrometry for translational proteomics: progress and clinical implications. *Genome medicine*. 2012;4:63.
- [29] Di Girolamo F, Lante I, Muraca M, Putignani L. The Role of Mass Spectrometry in the "Omics" Era. *Current organic chemistry*. 2013;17:2891-905.
- [30] Cunningham R, Ma D, Li L. Mass Spectrometry-based Proteomics and Peptidomics for Systems Biology and Biomarker Discovery. *Frontiers in biology*. 2012;7:313-35.
- [31] Chen Y, Chan CK, Kerishnan JP, Lau YL, Wong YL, Gopinath SC. Identification of circulating biomarkers in sera of Plasmodium knowlesi-infected malaria patients--comparison against Plasmodium vivax infection. *BMC infectious diseases*. 2015;15:49.
- [32] Mu AK, Bee PC, Lau YL, Chen Y. Identification of protein markers in patients infected with Plasmodium knowlesi, Plasmodium falciparum and Plasmodium vivax. *International journal of molecular sciences*. 2014;15:19952-61.
- [33] Ndao M, Spithill TW, Caffrey R, Li H, Podust VN, Perichon R, et al. Identification of novel diagnostic serum biomarkers for Chagas' disease in asymptomatic subjects by mass spectrometric profiling. *J Clin Microbiol*. 2010;48:1139-49.
- [34] Kailasa SK, Wu HF. Proteomic profiling by nanomaterials-based matrix-assisted laser desorption/ionization mass spectrometry for high-resolution data and novel protein information directly from biological samples. *Methods in molecular biology (Clifton, NJ)*. 2015;1295:479-96.

- [35] Adaszek L, Banach T, Bartnicki M, Winiarczyk D, Lyp P, Winiarczyk S. Application the mass spectrometry MALDI-TOF technique for detection of *Babesia canis canis* infection in dogs. *Parasitology research*. 2014;113:4293-5.
- [36] Calderaro A, Piergianni M, Buttrini M, Montecchini S, Piccolo G, Gorrini C, et al. MALDI-TOF mass spectrometry for the detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar*. *PLoS one*. 2015;10:e0122448.
- [37] Minerva L, Ceulemans A, Baggerman G, Arckens L. MALDI MS imaging as a tool for biomarker discovery: methodological challenges in a clinical setting. *Proteomics Clinical applications*. 2012;6:581-95.
- [38] Arafah K, Longuespee R, Desmons A, Kerdraon O, Fournier I, Salzet M. Lipidomics for clinical diagnosis: Dye-Assisted Laser Desorption/Ionization (DALDI) method for lipids detection in MALDI mass spectrometry imaging. *Omics : a journal of integrative biology*. 2014;18:487-98.
- [39] Uhlmann KR, Gibb S, Kalkhof S, Arroyo-Abad U, Schulz C, Hoffmann B, et al. Species determination of *Culicoides* biting midges via peptide profiling using matrix-assisted laser desorption ionization mass spectrometry. *Parasites & vectors*. 2014;7:392.
- [40] Fotso Fotso A, Mediannikov O, Diatta G, Almeras L, Flaudrops C, Parola P, et al. MALDI-TOF mass spectrometry detection of pathogens in vectors: the *Borrelia crocidurae*/*Ornithodoros sonrai* paradigm. *PLoS neglected tropical diseases*. 2014;8:e2984.
- [41] Ndao M, Spithill TW, Caffrey R, Li H, Podust VN, Perichon R, et al. Identification of novel diagnostic serum biomarkers for chagas' disease in asymptomatic subjects by mass spectrometric profiling. *Journal of Clinical Microbiology*. 2010;48:1139-49.
- [42] Wagner G, Eiko Yamanaka L, Moura H, Denardin Lückemeyer D, Schindwein AD, Hermes Stoco P, et al. The *Trypanosoma rangeli* trypomastigote surfaceome reveals novel proteins and targets for specific diagnosis. *Journal of proteomics*. 2013;82:52-63.
- [43] Tiberti N, Hainard A, Lejon V, Robin X, Ngoyi DM, Turck N, et al. Discovery and verification of osteopontin and Beta-2-microglobulin as promising markers for staging human African trypanosomiasis. *Molecular & cellular proteomics : MCP*. 2010;9:2783-95.
- [44] Robijn MLM, Planken J, Kornelis D, Hokke CH, Deelder AM. Mass spectrometric detection of urinary oligosaccharides as markers of *Schistosoma mansoni* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2008;102:79-83.
- [45] Garcia-Perez I, Earll ME, Angulo S, Barbas C, Legido-Quigley C. Chemometric analysis of urine fingerprints acquired by liquid chromatography-mass spectrometry and capillary electrophoresis: application to the schistosomiasis mouse model. *Electrophoresis*. 2010;31:2349-55.
- [46] Park YH, Shi YP, Liang B, Medriano CAD, Jeon YH, Torres E, et al. High-resolution metabolomics to discover potential parasite-specific biomarkers in a

- Plasmodium falciparum* erythrocytic stage culture system. *Malaria journal*. 2015;14:122.
- [47] Tritten L, Keiser J, Godejohann M, Utzinger J, Vargas M, Beckonert O, et al. Metabolic profiling framework for discovery of candidate diagnostic markers of malaria. *Scientific reports*. 2013;3:2769.
- [48] Zocevic A, Carmi-Leroy A, Sautereau J, d'Alayer J, Lenormand P, Rousselle J-C, et al. New markers in *Anopheles gambiae* salivary glands after *Plasmodium berghei* infection. *Vector borne and zoonotic diseases (Larchmont, NY)*. 2013;13:119-27.
- [49] Ahn C-S, Han X, Bae Y-A, Ma X, Kim J-T, Cai H, et al. Alteration of immunoproteome profile of *Echinococcus granulosus* hydatid fluid with progression of cystic echinococcosis. *Parasites & vectors*. 2015;8:10.
- [50] Wampfler PB, Tosevski V, Nanni P, Spycher C, Hehl AB. Proteomics of secretory and endocytic organelles in *Giardia lamblia*. *PloS one*. 2014;9:e94089.
- [51] Ali IKM, Haque R, Siddique A, Kabir M, Sherman NE, Gray SA, et al. Proteomic analysis of the cyst stage of *Entamoeba histolytica*. *PLoS neglected tropical diseases*. 2012;6:e1643.
- [52] Oakes RD, Kurian D, Bromley E, Ward C, Lal K, Blake DP, et al. The rhoptry proteome of *Eimeria tenella* sporozoites. *International journal for parasitology*. 2013;43:181-8.
- [53] Saadatnia G, Mohamed Z, Ghaffarifar F, Osman E, Moghadam ZK, Noordin R. *Toxoplasma gondii* excretory secretory antigenic proteins of diagnostic potential. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*. 2012;120:47-55.
- [54] Wang L, Cui J, Hu DD, Liu RD, Wang ZQ. Identification of early diagnostic antigens from major excretory-secretory proteins of *Trichinella spiralis* muscle larvae using immunoproteomics. *Parasites & vectors*. 2014;7:40.
- [55] Deckers N, Dorny P, Kanobana K, Vercruyse J, Gonzalez AE, Ward B, et al. Use of ProteinChip technology for identifying biomarkers of parasitic diseases: the example of porcine cysticercosis (*Taenia solium*). *Experimental parasitology*. 2008;120:320-9.
- [56] Magalhães-Junior JT, Mesquita PRR, Oliveira WFdS, Oliveira FS, Franke CR, Rodrigues FdM, et al. Identification of biomarkers in the hair of dogs: new diagnostic possibilities in the study and control of visceral leishmaniasis. *Analytical and bioanalytical chemistry*. 2014;406:6691-700.
- [57] Abeijon C, Kashino SS, Silva FO, Costa DL, Fujiwara RT, Costa CHN, et al. Identification and diagnostic utility of *Leishmania infantum* proteins found in urine samples from patients with visceral leishmaniasis. *Clinical and vaccine immunology : CVI*. 2012;19:935-43.
- [58] Ramón-Luing La, Rendón-Gandarilla FJ, Cárdenas-Guerra RE, Rodríguez-Cabrera Na, Ortega-López J, Avila-González L, et al. Immunoproteomics of the active degradome to identify biomarkers for *Trichomonas vaginalis*. *Proteomics*. 2010;10:435-44.

- [59] Goldfinch GM, Smith WD, Imrie L, McLean K, Inglis NF, Pemberton AD. The proteome of gastric lymph in normal and nematode infected sheep. *Proteomics*. 2008;8:1909-18.
- [60] Ye H, Gemperline E, Li L. A vision for better health: mass spectrometry imaging for clinical diagnostics. *Clinica chimica acta; international journal of clinical chemistry*. 2013;420:11-22.
- [61] Wolk DM, Kaleta EJ, Wysocki VH. PCR-electrospray ionization mass spectrometry: the potential to change infectious disease diagnostics in clinical and public health laboratories. *The Journal of molecular diagnostics: JMD*. 2012;14:295-304.
- [62] Eshoo MW, Crowder CD, Carolan HE, Rounds MA, Ecker DJ, Haag H, et al. Broad-range survey of tick-borne pathogens in Southern Germany reveals a high prevalence of *Babesia microti* and a diversity of other tick-borne pathogens. *Vector Borne Zoonotic Dis*. 2014;14:584-91.
- [63] Parker CE, Borchers CH. Mass spectrometry based biomarker discovery, verification, and validation--quality assurance and control of protein biomarker assays. *Molecular oncology*. 2014;8:840-58.
- [64] Timm T, Lenz C, Merkel D, Sadiffo C, Grabitzki J, Klein J, et al. Detection and site localization of phosphorylcholine-modified peptides by NanoLC-ESI-MS/MS using precursor ion scanning and multiple reaction monitoring experiments. *Journal of the American Society for Mass Spectrometry*. 2015;26:460-71.
- [65] Lv H, Hung CS, Chaturvedi KS, Hooton TM, Henderson JP. Development of an integrated metabolomic profiling approach for infectious diseases research. *The Analyst*. 2011;136:4752-63.
- [66] Garcia-Perez I, Earll ME, Angulo S, Barbas C, Legido-Quigley C. Chemometric analysis of urine fingerprints acquired by liquid chromatography-mass spectrometry and capillary electrophoresis: application to the schistosomiasis mouse model. *Electrophoresis*. 2010;31:2349-55.
- [67] Canuto GA, Castilho-Martins EA, Tavares M, Lopez-Gonzalvez A, Rivas L, Barbas C. CE-ESI-MS metabolic fingerprinting of *Leishmania* resistance to antimony treatment. *Electrophoresis*. 2012;33:1901-10.
- [68] Ng Hublin JS, Ryan U, Trengove R, Maker G. Metabolomic profiling of faecal extracts from *Cryptosporidium parvum* infection in experimental mouse models. *PloS one*. 2013;8:e77803.
- [69] Kobeissy FH, Gulbakan B, Alawieh A, Karam P, Zhang Z, Guingab-Cagmat JD, et al. Post-genomics nanotechnology is gaining momentum: nanoproteomics and applications in life sciences. *Omics : a journal of integrative biology*. 2014;18:111-31.
- [70] Manesse M, Phillips AF, LaFratta CN, Palacios MA, Hayman RB, Walt DR. Dynamic microbead arrays for biosensing applications. *Lab on a chip*. 2013;13:2153-60.
- [71] Natesan M, Ulrich RG. Protein microarrays and biomarkers of infectious disease. *International journal of molecular sciences*. 2010;11:5165-83.

- [72] Manzano-Roman R, Dasilva N, Diez P, Diaz-Martin V, Perez-Sanchez R, Orfao A, et al. Protein arrays as tool for studies at the host-pathogen interface. *Journal of proteomics*. 2013;94:387-400.
- [73] Carmona SJ, Nielsen M, Schafer-Nielsen C, Mucci J, Altchek J, Balouz V, et al. Towards high-throughput immunomics for infectious diseases: use of next-generation peptide microarrays for rapid discovery and mapping of antigenic determinants. *Molecular & cellular proteomics : MCP*. 2015.
- [74] Hong CC, Chen CP, Horng JC, Chen SY. Point-of-care protein sensing platform based on immuno-like membrane with molecularly-aligned nanocavities. *Biosensors & bioelectronics*. 2013;50:425-30.
- [75] Obishakin E, Stijlemans B, Santi-Rocca J, Vandenberghe I, Devreese B, Muldermans S, et al. Generation of a nanobody targeting the paraflagellar rod protein of trypanosomes. *PloS one*. 2014;9:e115893.
- [76] Caljon G, Hussain S, Vermeiren L, Van Den Abbeele J. Description of a nanobody-based competitive immunoassay to detect tsetse fly exposure. *PLoS neglected tropical diseases*. 2015;9:e0003456.
- [77] Muthana SM, Gildersleeve JC. Glycan microarrays: powerful tools for biomarker discovery. *Cancer biomarkers : section A of Disease markers*. 2014;14:29-41.
- [78] Anish C, Martin CE, Wahlbrink A, Bogdan C, Ntais P, Antoniou M, et al. Immunogenicity and diagnostic potential of synthetic antigenic cell surface glycans of *Leishmania*. *ACS chemical biology*. 2013;8:2412-22.
- [79] Aranzamendi C, Tefsen B, Jansen M, Chiumiento L, Bruschi F, Kortbeek T, et al. Glycan microarray profiling of parasite infection sera identifies the LDNF glycan as a potential antigen for serodiagnosis of trichinellosis. *Experimental parasitology*. 2011;129:221-6.
- [80] Marcilla A, Martin-Jaular L, Trelis M, de Menezes-Neto A, Osuna A, Bernal D, et al. Extracellular vesicles in parasitic diseases. *Journal of extracellular vesicles*. 2014;3:25040.
- [81] Atayde VD, Aslan H, Townsend S, Hassani K, Kamhawi S, Olivier M. Exosome Secretion by the Parasitic Protozoan *Leishmania* within the Sand Fly Midgut. *Cell reports*. 2015;13:957-67.
- [82] Inal JM, Kosgodage U, Azam S, Stratton D, Antwi-Baffour S, Lange S. Blood/plasma secretome and microvesicles. *Biochimica et biophysica acta*. 2013;1834:2317-25.
- [83] Manzano-Roman R, Siles-Lucas M. MicroRNAs in parasitic diseases: potential for diagnosis and targeting. *Molecular and biochemical parasitology*. 2012;186:81-6.
- [84] Carrara S, Ghoreishizadeh S, Olivo J, Taurino I, Baj-Rossi C, Cavallini A, et al. Fully integrated biochip platforms for advanced healthcare. *Sensors (Basel, Switzerland)*. 2012;12:11013-60.
- [85] Battle KN, Uba FI, Soper SA. Microfluidics for the analysis of membrane proteins: how do we get there? *Electrophoresis*. 2014;35:2253-66.
- [86] Wilson DH, Rissin DM, Kan CW, Fournier DR, Piech T, Campbell TG, et al. The Simoa HD-1 Analyzer: A Novel Fully Automated Digital Immunoassay

- Analyzer with Single-Molecule Sensitivity and Multiplexing. *Journal of laboratory automation*. 2015; Jun 15. pii: 2211068215589580.
- [87] Sin ML, Mach KE, Wong PK, Liao JC. Advances and challenges in biosensor-based diagnosis of infectious diseases. *Expert review of molecular diagnostics*. 2014;14:225-44.
- [88] Willison KR, Klug DR. Quantitative single cell and single molecule proteomics for clinical studies. *Current opinion in biotechnology*. 2013;24:745-51.
- [89] Mu B, Zhang J, McNicholas TP, Reuel NF, Kruss S, Strano MS. Recent advances in molecular recognition based on nanoengineered platforms. *Accounts of chemical research*. 2014;47:979-88.
- [90] Dias AD, Kingsley DM, Corr DT. Recent advances in bioprinting and applications for biosensing. *Biosensors*. 2014;4:111-36.
- [91] Otranto D. Diagnostic challenges and the unwritten stories of dog and cat parasites. *Veterinary parasitology*. 2015;212:54-61.
- [92] Muwonge H, Kikomeko S, Sembajje LF, Seguya A, Namugwanya C. How Reliable Are Hematological Parameters in Predicting Uncomplicated Malaria in an Endemic Region? *ISRN tropical medicine*. 2013;2013:1-9.
- [93] Elbashir HM, Salih MM, Elhassan EM, Mohammed AA, Elbashir MI, Adam I. Polymerase chain reaction and histology in diagnosis of placental malaria in an area of unstable malaria transmission in Central Sudan. *Diagnostic pathology*. 2011;6:128.
- [94] Graffeo R, Masucci L, Bugli F, Damiano F, Pinnetti C, Federico G, et al. Multiple malaria infection in a pregnant woman from Nigeria: detection by multiplex PCR. *The new microbiologica*. 2008;31:565-7.
- [95] Rogerson SJ, Mkundika P, Kanjala MK. Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology. *J Clin Microbiol*. 2003;41:1370-4.
- [96] Diseases WHOobotSPfRaTiT. Malaria Rapid Diagnostic Test Performance. Results of WHO product testing of malaria RDTs: Round 3 (2010-2011). 2011.
- [97] Mouatcho JC, Goldring JP. Malaria rapid diagnostic tests: challenges and prospects. *Journal of medical microbiology*. 2013;62:1491-505.
- [98] Solca Mda S, Bastos LA, Guedes CE, Bordoni M, Borja LS, Larangeira DF, et al. Evaluating the accuracy of molecular diagnostic testing for canine visceral leishmaniasis using latent class analysis. *PloS one*. 2014;9:e103635.
- [99] Abass E, Kang C, Martinkovic F, Semiao-Santos SJ, Sundar S, Walden P, et al. Heterogeneity of *Leishmania donovani* parasites complicates diagnosis of visceral leishmaniasis: comparison of different serological tests in three endemic regions. *PloS one*. 2015;10:e0116408.
- [100] Pereira VR, Reis Lde C, Souza Mde A, de Oliveira AP, de Brito ME, Lage PS, et al. Evaluation of anti-lived and anti-fixed *Leishmania (Viannia) braziliensis* promastigote IgG antibodies detected by flow cytometry for diagnosis and post-therapeutic cure assessment in localized cutaneous leishmaniasis. *Diagnostic microbiology and infectious disease*. 2012;74:292-8.

- [101] Menezes-Souza D, Mendes TA, Nagem RA, Santos TT, Silva AL, Santoro MM, et al. Mapping B-cell epitopes for the peroxidoxin of *Leishmania (Viannia) braziliensis* and its potential for the clinical diagnosis of tegumentary and visceral leishmaniasis. *PloS one*. 2014;9:e99216.
- [102] Bua J, Volta BJ, Perrone AE, Scollo K, Velazquez EB, Ruiz AM, et al. How to improve the early diagnosis of *Trypanosoma cruzi* infection: relationship between validated conventional diagnosis and quantitative DNA amplification in congenitally infected children. *PLoS neglected tropical diseases*. 2013;7:e2476.
- [103] Afonso AM, Ebell MH, Tarleton RL. A systematic review of high quality diagnostic tests for Chagas disease. *PLoS neglected tropical diseases*. 2012;6:e1881.
- [104] Machado-de-Assis GF, Silva AR, Do Bem VA, Bahia MT, Martins-Filho OA, Dias JC, et al. Posttherapeutic cure criteria in Chagas' disease: conventional serology followed by supplementary serological, parasitological, and molecular tests. *Clin Vaccine Immunol*. 2012;19:1283-91.
- [105] Brasil PE, De Castro L, Hasslocher-Moreno AM, Sangenis LH, Braga JU. ELISA versus PCR for diagnosis of chronic Chagas disease: systematic review and meta-analysis. *BMC infectious diseases*. 2010;10:337.
- [106] Khammari I, Saghrouni F, Lakhal S, Bouratbine A, Ben Said M, Boukadida J. A new IgG immunoblot kit for diagnosis of toxoplasmosis in pregnant women. *The Korean journal of parasitology*. 2014;52:493-9.
- [107] Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends in parasitology*. 2009;25:151-6.
- [108] Cavalcanti MG, Silva LF, Peralta RH, Barreto MG, Peralta JM. Schistosomiasis in areas of low endemicity: a new era in diagnosis. *Trends in parasitology*. 2013;29:75-82.
- [109] Hernandez-Gonzalez A, Santivanez S, Garcia HH, Rodriguez S, Munoz S, Ramos G, et al. Improved serodiagnosis of cystic echinococcosis using the new recombinant 2B2t antigen. *PLoS neglected tropical diseases*. 2012;6:e1714.
- [110] Gottstein B, Wang J, Blagosklonov O, Grenouillet F, Millon L, Vuitton DA, et al. *Echinococcus* metacestode: in search of viability markers. *Parasite (Paris, France)*. 2014;21:63.
- [111] Conraths FJ, Deplazes P. *Echinococcus multilocularis*: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. *Veterinary parasitology*. 2015;213(3-4):149-61.
- [112] Wong SS, Fung KS, Chau S, Poon RW, Wong SC, Yuen KY. Molecular diagnosis in clinical parasitology: when and why? *Experimental biology and medicine (Maywood, NJ)*. 2014;239:1443-60.
- [113] Machado FS, Mukherjee S, Weiss LM, Tanowitz HB, Ashton AW. Bioactive lipids in *Trypanosoma cruzi* infection. *Advances in parasitology*. 2011;76:1-31.
- [114] Mortimer L, Moreau F, Cornick S, Chadee K. Gal-lectin-dependent contact activates the inflammasome by invasive *Entamoeba histolytica*. *Mucosal immunology*. 2014;7:829-41.

- [115] Nagajyothi F, Machado FS, Burleigh BA, Jelicks LA, Scherer PE, Mukherjee S, et al. Mechanisms of *Trypanosoma cruzi* persistence in Chagas disease. *Cellular microbiology*. 2012;14:634-43.
- [116] Huang H, Mackeen MM, Cook M, Oriero E, Locke E, Thezenas ML, et al. Proteomic identification of host and parasite biomarkers in saliva from patients with uncomplicated *Plasmodium falciparum* malaria. *Malaria journal*. 2012;11:178.
- [117] Silva-Moraes V, Ferreira JM, Coelho PM, Grenfell RF. Biomarkers for schistosomiasis: towards an integrative view of the search for an effective diagnosis. *Acta tropica*. 2014;132:75-9.
- [118] Goulart LR, Vieira CU, Freschi AP, Capparelli FE, Fujimura PT, Almeida JF, et al. Biomarkers for serum diagnosis of infectious diseases and their potential application in novel sensor platforms. *Critical reviews in immunology*. 2010;30:201-22.
- [119] Du E, Ha S, Diez-Silva M, Dao M, Suresh S, Chandrakasan AP. Electric impedance microflow cytometry for characterization of cell disease states. *Lab on a chip*. 2013;13:3903-9.
- [120] Nam J, Huang H, Lim H, Lim C, Shin S. Magnetic separation of malaria-infected red blood cells in various developmental stages. *Analytical chemistry*. 2013;85:7316-23.
- [121] Kataoka M, Abe K, Hashimoto Y, Yamamura S, Yatsushiro S. [Development of microchips for the analysis of biomarkers in blood]. *Rinsho byori The Japanese journal of clinical pathology*. 2012;60:1094-100.
- [122] Taylor BJ, Howell A, Martin KA, Manage DP, Gordy W, Campbell SD, et al. A lab-on-chip for malaria diagnosis and surveillance. *Malaria journal*. 2014;13:179.
- [123] Geislinger TM, Chan S, Moll K, Wixforth A, Wahlgren M, Franke T. Label-free microfluidic enrichment of ring-stage *Plasmodium falciparum*-infected red blood cells using non-inertial hydrodynamic lift. *Malaria journal*. 2014;13:375.
- [124] Hsu YH, Lu P, Coleman JL, Tang WC. A microfluidic platform to isolate avian erythrocytes infected with *Plasmodium gallinaceum* malaria parasites based on surface morphological changes. *Biomedical microdevices*. 2011;13:995-1004.
- [125] Jubery TZ, Dutta P. A new design for efficient dielectrophoretic separation of cells in a microdevice. *Electrophoresis*. 2013;34:643-50.
- [126] Reboud J, Bourquin Y, Wilson R, Pall GS, Jiwaji M, Pitt AR, et al. Shaping acoustic fields as a toolset for microfluidic manipulations in diagnostic technologies. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;109:15162-7.
- [127] Herricks T, Seydel KB, Molyneux M, Taylor T, Rathod PK. Estimating physical splenic filtration of *Plasmodium falciparum*-infected red blood cells in malaria patients. *Cellular microbiology*. 2012;14:1880-91.
- [128] Guo Q, Reiling SJ, Rohrbach P, Ma H. Microfluidic biomechanical assay for red blood cells parasitized by *Plasmodium falciparum*. *Lab on a chip*. 2012;12:1143-50.

- [129] Lee SS, Yim Y, Ahn KH, Lee SJ. Extensional flow-based assessment of red blood cell deformability using hyperbolic converging microchannel. *Biomedical microdevices*. 2009;11:1021-7.
- [130] Bow H, Pivkin IV, Diez-Silva M, Goldfless SJ, Dao M, Niles JC, et al. A microfabricated deformability-based flow cytometer with application to malaria. *Lab on a chip*. 2011;11:1065-73.
- [131] Hou HW, Bhagat AA, Chong AG, Mao P, Tan KS, Han J, et al. Deformability based cell margination--a simple microfluidic design for malaria-infected erythrocyte separation. *Lab on a chip*. 2010;10:2605-13.
- [132] Handayani S, Chiu DT, Tjitra E, Kuo JS, Lampah D, Kenangalem E, et al. High deformability of Plasmodium vivax-infected red blood cells under microfluidic conditions. *The Journal of infectious diseases*. 2009;199:445-50.
- [133] Grover WH, Bryan AK, Diez-Silva M, Suresh S, Higgins JM, Manalis SR. Measuring single-cell density. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108:10992-6.
- [134] Bishara W, Sikora U, Mudanyali O, Su TW, Yaglidere O, Luckhart S, et al. Holographic pixel super-resolution in portable lensless on-chip microscopy using a fiber-optic array. *Lab on a chip*. 2011;11:1276-9.
- [135] Puskar L, Tuckermann R, Frosch T, Popp J, Ly V, McNaughton D, et al. Raman acoustic levitation spectroscopy of red blood cells and Plasmodium falciparum trophozoites. *Lab on a chip*. 2007;7:1125-31.
- [136] Orban A, Butykai A, Molnar A, Prohle Z, Fulop G, Zelles T, et al. Evaluation of a novel magneto-optical method for the detection of malaria parasites. *PloS one*. 2014;9:e96981.
- [137] Juul S, Nielsen CJ, Labouriau R, Roy A, Tesauro C, Jensen PW, et al. Droplet microfluidics platform for highly sensitive and quantitative detection of malaria-causing Plasmodium parasites based on enzyme activity measurement. *ACS nano*. 2012;6:10676-83.
- [138] Warkiani ME, Tay AK, Khoo BL, Xiaofeng X, Han J, Lim CT. Malaria detection using inertial microfluidics. *Lab on a chip*. 2015;15:1101-9.
- [139] Laki AJN, G.Z. ; Ivan, K. ; Furjes, P. ; Jacso, O. ; Fok, E. ; Civera, P. Integrated microcapillary system for microfluidic parasite analysis *Fac of Inf Technol & Bionics*. Budapest, Hungary IEEE; 2013. p. 118-21.
- [140] Hu C, Kearn J, Urwin P, Lilley C, V OC, Holden-Dye L, et al. StyletChip: a microfluidic device for recording host invasion behaviour and feeding of plant parasitic nematodes. *Lab on a chip*. 2014;14:2447-55.
- [141] Teles FS. Biosensors and rapid diagnostic tests on the frontier between analytical and clinical chemistry for biomolecular diagnosis of dengue disease: a review. *Analytica chimica acta*. 2011;687:28-42.
- [142] Cabral-Miranda G, de Jesus JR, Oliveira PR, Britto GS, Pontes-de-Carvalho LC, Dutra RF, et al. Detection of parasite antigens in Leishmania infantum-infected spleen tissue by monoclonal antibody-, piezoelectric-based immunosensors. *The Journal of parasitology*. 2014;100:73-8.

- [143] Jeon W, Lee S, Manjunatha DH, Ban C. A colorimetric aptasensor for the diagnosis of malaria based on cationic polymers and gold nanoparticles. *Analytical biochemistry*. 2013;439:11-6.
- [144] Tesauro C, Juul S, Arno B, Nielsen CJ, Fiorani P, Frohlich RF, et al. Specific detection of topoisomerase I from the malaria causing *P. falciparum* parasite using isothermal rolling circle amplification. *Conference proceedings : Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference*. 2012;2012:2416-9.
- [145] Potipitak T, Ngrenngarmert W, Promptmas C, Chomean S, Ittarat W. Diagnosis and genotyping of *Plasmodium falciparum* by a DNA biosensor based on quartz crystal microbalance (QCM). *Clinical chemistry and laboratory medicine : CCLM / FESCC*. 2011;49:1367-73.
- [146] Lee SA, Leitao R, Zheng G, Yang S, Rodriguez A, Yang C. Color capable sub-pixel resolving optofluidic microscope and its application to blood cell imaging for malaria diagnosis. *PloS one*. 2011;6:e26127.
- [147] Angus SV, Kwon HJ, Yoon JY. Field-deployable and near-real-time optical microfluidic biosensors for single-oocyst-level detection of *Cryptosporidium parvum* from field water samples. *Journal of environmental monitoring : JEM*. 2012;14:3295-304.
- [148] Park CK, Kang CD, Sim SJ. Non-labeled detection of waterborne pathogen *Cryptosporidium parvum* using a polydiacetylene-based fluorescence chip. *Biotechnology journal*. 2008;3:687-93.
- [149] Taguchi T, Arakaki A, Takeyama H, Haraguchi S, Yoshino M, Kaneko M, et al. Detection of *Cryptosporidium parvum* oocysts using a microfluidic device equipped with the SUS micromesh and FITC-labeled antibody. *Biotechnology and bioengineering*. 2007;96:272-80.
- [150] Wang S, Yin T, Zeng S, Che H, Yang F, Chen X, et al. A piezoelectric immunosensor using hybrid self-assembled monolayers for detection of *Schistosoma japonicum*. *PloS one*. 2012;7:e30779.
- [151] Zeng S, Tian Z, Che H, Yang H, Chen X, Feng Q, et al. Novel printed electrode immunosensors for *Schistosoma japonicum*. *Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences*. 2012;37:541-8.
- [152] Wen Z, Wang S, Wu Z, Shen G. A novel liquid-phase piezoelectric immunosensor for detecting *Schistosoma japonicum* circulating antigen. *Parasitology international*. 2011;60:301-6.
- [153] Linder E, Grote A, Varjo S, Linder N, Lebbad M, Lundin M, et al. On-chip imaging of *Schistosoma haematobium* eggs in urine for diagnosis by computer vision. *PLoS neglected tropical diseases*. 2013;7:e2547.
- [154] Xu S, Mutharasan R. Rapid and sensitive detection of *Giardia lamblia* using a piezoelectric cantilever biosensor in finished and source waters. *Environmental science & technology*. 2010;44:1736-41.

- [155] Lee LM, Cui X, Yang C. The application of on-chip optofluidic microscopy for imaging *Giardia lamblia* trophozoites and cysts. *Biomedical microdevices*. 2009;11:951-8.
- [156] Ribone ME, Belluzo MS, Pagani D, Marcipar IS, Lagier CM. Amperometric bioelectrode for specific human immunoglobulin G determination: optimization of the method to diagnose American trypanosomiasis. *Analytical biochemistry*. 2006;350:61-70.
- [157] Pak BJ, Vasquez-Camargo F, Kalinichenko E, Chiodini PL, Nutman TB, Tanowitz HB, et al. Development of a rapid serological assay for the diagnosis of strongyloidiasis using a novel diffraction-based biosensor technology. *PLoS neglected tropical diseases*. 2014;8:e3002.
- [158] Grewal YS, Shiddiky MJ, Spadafora LJ, Cangelosi GA, Trau M. Nano-yeast-scFv probes on screen-printed gold electrodes for detection of *Entamoeba histolytica* antigens in a biological matrix. *Biosensors & bioelectronics*. 2014;55:417-22.
- [159] Nascimento E, Silva T, Oliva A. Identification, characterization and manipulation of *Babesia-bovis*-infected red blood cells using microfluidics technology. *Parassitologia*. 2007;49 Suppl 1:45-52.
- [160] Cramp AP, Sohn JH, James PJ. Detection of cutaneous myiasis in sheep using an 'electronic nose'. *Veterinary parasitology*. 2009;166:293-8.
- [161] Lam B, Das J, Holmes RD, Live L, Sage A, Sargent EH, et al. Solution-based circuits enable rapid and multiplexed pathogen detection. *Nature communications*. 2013;4:2001.
- [162] Tan JJ, Capozzoli M, Sato M, Watthanaworawit W, Ling CL, Mauduit M, et al. An integrated lab-on-chip for rapid identification and simultaneous differentiation of tropical pathogens. *PLoS neglected tropical diseases*. 2014;8:e3043.
- [163] Lockery SR, Hulme SE, Roberts WM, Robinson KJ, Laromaine A, Lindsay TH, et al. A microfluidic device for whole-animal drug screening using electrophysiological measures in the nematode *C. elegans*. *Lab on a chip*. 2012;12:2211-20.
- [164] Lycke R, Parashar A, Pandey S. Microfluidics-enabled method to identify modes of *Caenorhabditis elegans* paralysis in four anthelmintics. *Biomicrofluidics*. 2013;7:64103.
- [165] Carr JA, Parashar A, Gibson R, Robertson AP, Martin RJ, Pandey S. A microfluidic platform for high-sensitivity, real-time drug screening on *C. elegans* and parasitic nematodes. *Lab on a chip*. 2011;11:2385-96.
- [166] Horning MP, Delahunt CB, Singh SR, Garing SH, Nichols KP. A paper microfluidic cartridge for automated staining of malaria parasites with an optically transparent microscopy window. *Lab on a chip*. 2014;14:2040-6.
- [167] Connelly JT, Baumner AJ. Biosensors for the detection of waterborne pathogens. *Analytical and bioanalytical chemistry*. 2012;402:117-27.

- [168] Lee SA, Erath J, Zheng G, Ou X, Willems P, Eichinger D, et al. Imaging and identification of waterborne parasites using a chip-scale microscope. *PloS one*. 2014;9:e89712.
- [169] Dibao-Dina A, Follet J, Ibrahim M, Vlandas A, Senez V. Electrical impedance sensor for quantitative monitoring of infection processes on HCT-8 cells by the waterborne parasite *Cryptosporidium*. *Biosensors & bioelectronics*. 2015;66:69-76.
- [170] Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, et al. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International journal of food microbiology*. 2010;139 Suppl 1:S3-15.
- [171] Dhama K, Rajagunalan S, Chakraborty S, Verma AK, Kumar A, Tiwari R, et al. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pakistan journal of biological sciences: PJBS*. 2013;16:1076-85.

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.

Table 1. New potential biomarkers identified in host-parasite models found by mass spectrometry techniques.

Disease/Parasite	Biomarkers	Source	Method	Features/Details	References
<i>Trypanosoma cruzi</i>	C3a anaphylatoxin, apolipoprotein A fibronectin	Serum	Mass Spectrometric Profiling by SELDI-TOF	Useful for subjects with latent Chagas Disease	[41]
<i>T. rangeli</i>	T. rangeli GP63-related protein and an FCaBP protein	Serum	MS/MS analysis	Proteins from trypomastigote surfome	[42]
<i>T. brucei gambiense</i>	Osteopontin and β -2-microglobulin	Cerebrospinal fluid	MALDI TOF-TOF MS	Good candidates for the development of a test for staging patients	[43]
<i>Schistosoma mansoni</i>	Fuco-oligosaccharides that are produced by schistosome eggs	Urine	MALDI-TOF	Oligosaccharides detected in infection urine are shown to be excreted by live eggs and not by worms	[44]
	Phenyl acetyl glycine (PAG)		UPLC-MS	PAG is a statistically discriminant metabolite which can provide important information regarding the disease	[45]
<i>Plasmodium falciparum</i>	Metabolites between supernatants from Plasmodium: 3- methylindole, succinylacetone, S-methyl-L-thiocitrulline, O- arachidonoyl glycidol	In vitro cultured samples	High resolution metabolomics (HRM): C18 liquid chromatography coupled with Fourier-Transform Mass Spectrometry (FTMS)	Low molecular-weight metabolites for future development of non- invasive malaria diagnostic tools	[46]
	Urinary metabolites: UK1, UK2	Urine	Nuclear magnetic resonance (NMR) profiling followed by LC-MS	Two unique structurally related urinary candidate biomarkers have not been described so far in the eukaryotic organism.	[47]
<i>P. berghei</i>	Proteins: cE5, B3VDI9_ANOGA, and AGAP008216-PA	Salivary gland samples from <i>Anopheles gambiae</i>	SELDI-TOF-MS	Markers involved in blood feeding	[48]
<i>Echinococcus spp</i>	Antigen B-related molecules (EgAgB; EgAgB1-5)	Sera	MALDI-TOF-MS	Immunodominant epitopes changed as the disease progresses	[49]
<i>Giardia lamblia</i>	Encystation-specific vesicles (ESVs) and endocytic organelles termed peripheral vesicles (PVs)	Microsome fractions derived from trophozoites	Combining flow cytometry-based organelle sorting with In silico filtration of mass	Proteins from Secretory and Endocytic Organelles:	[50]

<i>Entamoeba histolytica</i>	Cyst-wall specific glycoproteins Jacob, Jessie and chitinase	Fecal specimens	spectrometry data LC-MS/MS mass spectrometer	Promise as diagnostic targets	[51]
<i>Eimeria</i> /Coccidiosis	Proteins from the neck region (rhoptry neck proteins, RON): RON2L1, RON2L2	Serum	Gel LC- MS/MS	From enriched rhoptry fractions isolated from the sporozoite stage	[52]
<i>Toxoplasma gondii</i>	Excretory secretory antigen (ESA): microneme protein 10 and dense granule protein 7, phosphoglycerate mutase 1	Serum	MALDI-TOF-TOF	From tachyzoites	[53]
<i>Trichinella spiralis</i>	Excretory-secretory (ES) proteins of muscle larvae (ML)	Serum	MALDI-TOF	Come mainly from the excretory granules of the stichosome and the cuticles membrane	[54]
Porcine cysticercosis (<i>Taenia solium</i>)	Clusterin, lecithin-cholesterol acyltransferase, vitronectin, haptoglobin and apolipoprotein A-I	Serum	SELDI-TOF technology	Detection of viable cysts (active disease)	[55]
<i>Leishmania infantum</i>	Profile of the volatile organic (VOCs) emitted	Hair sample	Solid-phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS)	Significant variations between healthy dogs (G1) and infected dogs (G2+G3).	[56]
	Proteins Li-isd1, Li-txn1, Li-ntf2	Urine	RP-HPLC-MS	To distinguish active visceral leishmaniasis from asymptomatic infection	[57]
<i>Trichomonas vaginalis</i>	Papain-like (TvCP2, TvCP4, TvCP4-like, TvCPT), and one legumain-like (TvLEGU-1) cysteine proteinases(CPs)	Serum	MALDI-MS and ESI-LC-MS/MS	Antigen cocktail of recombinant proteinases that could increase the sensitivity and specificity for the immunodiagnosis	[58]
<i>Teladorsagia circumcincta</i> (parasitic nematode)	Gelsolin, α -1 b glycoprotein and haemopexin	Lymph formed from the interstitial fluid (sheeps)	MALDI-TOF and MS/MS analyses	The proteomic study of lymph has the potential to give new insights into local responses to infection	[59]

Table 2. Some key problems to diagnose parasitic diseases and encountered in existing diagnostics for a range of important parasitic species.

PARASITIC DISEASE	PROBLEMS RELATED TO THEIR DIAGNOSIS	SPECIES	DIAGNOSTIC METHOD	DEFICIENCIES/INADEQUACIES	REFERENCES
MALARIA	<p>-Conventional microscopy is the gold standard for malaria diagnosis. 50-100 parasites/μl of blood can be detected in a good lab. Sensitivity is only 500 parasites/μl in non-specialized labs. Requires expertise.</p> <p>-Great difficulty of establishing quality-assured microscopy in rural and resource-poor settings.</p> <p>-Field implementation of many techniques remains a problem.</p> <p>- Rapid diagnostic tests have good sensitivity for densities of <i>P. falciparum</i> greater than 500 parasites/μL. However, the World Health Organization recommends a lower sensitivity limit of detection for rapid diagnostic test for <i>P. falciparum</i> of 95% at a parasitaemia of 100P/μL.</p> <p>- Current tests are essentially qualitative and do not quantitate the risk of developing severe complication.</p> <p>- Most RDTs that detect multiple species do not differentiate non-<i>P. falciparum</i> species from each other, nor do they differentiate mixed infections of <i>P. falciparum</i> and non-<i>P. falciparum</i> from <i>P. falciparum</i> mono-infection.</p> <p>-Placental malaria poses a great diagnostic challenge, lack of accurate and sensitive diagnostic tool for malaria infections in pregnancy.</p>	<i>Plasmodium falciparum</i>	Hematological parameters (Analytical Biochemistry)	Unreliable laboratory indicators in acute uncomplicated malaria	[92]
			Nested PCR	Time-consuming, open to considerable risk of contamination, low cost-efficiency and low sensitivity and specificity in certain cases.	[93]
			Light microscopy	Their efficacy is affected by several key factors such as the level of parasitemia, among others.	[94]
			Histopathology	Frequently not available in most settings, relatively costly and labor intensive.	[95]
			Rapid diagnostic tests	The specificities, sensitivities, numbers of false positives, numbers of false negatives and temperature tolerances of these tests vary considerably	[96]
	Performance varies between lots and widely between similar products. Also varies the concentration in the blood of the protein to be detected.	[97]			
LEISHMANIASIS	<p>-Heterogeneity of <i>Leishmania</i> parasites complicates the diagnosis.</p> <p>-Proteins stage-specifically expressed and associated with virulence have a high antigenicity during the active disease phase.</p>	<i>Leishmania infantum/donovani</i>	Quantitative PCR	Invasive samples for accurate detection	[98]
			Serology	The specifications for VL diagnostic tests vary among the different endemic regions.	[99]

	<ul style="list-style-type: none"> -Antigens used in serology show a large number of cross reactions with other trypanosomatids. -Invasive parasitological methods currently used to identify infected Individuals. 	<i>Leishmania braziliensis</i>		No gold-standard test for tegumentary leishmaniasis, a combination of different diagnostic techniques is often necessary.	[110]
			Serology (ELISA)	Potential as an alternative method for confirmation.	[101]
TRYPANOSOMIASIS	<ul style="list-style-type: none"> -No symptoms in acute or chronic phase and once the immune response is established, parasite detection is very difficult. -Misdiagnosis between species. -There is currently no single reference standard test. -Methodological limitations, especially in sensitivity and specificity. The direct or parasitological tests have unacceptably low sensitivity in the chronic phase. -There is a need for tools that can identify patients cured shortly after specific treatment. Other needs include a marker for prognosis and early diagnosis of congenital transmission. -Failure to detect parasites in infected newborns at one month of age due to low sensitivity of the assays. 	<i>Trypanosoma cruzi</i>	INP micromethod	Parasite burden in some patients is below the detection limit	[102]
			Serological methods	Frequently display cross-reactivity against other pathogens and long term required for host seroreversion after the etiologic treatment of <i>T. cruzi</i> infection. Sensitivity and specificity have low accuracy.	[103,104]
			PCR	Controversial for chronic phase disease diagnosis.	[105]
TOXOPLASMOSIS	<ul style="list-style-type: none"> -Detection of oocysts is of little significance owing to short patency. The serological diagnosis of prenatal infection is difficult. -Low and focal distribution of parasites in the tissues or to the presence of non viable parasites. -Diagnosis of acute infection in human pregnancy is difficult since antibodies can be detected for a very long time after the acute phase. 	<i>Toxoplasma gondii</i>	Serological methods	Equivocal results with conventional serological techniques are not uncommon when <i>IgG</i> titers are close to the cut-off value of the test	[106]
SCHISTOSOMIASIS	<ul style="list-style-type: none"> -Microscopic examination of excreta is the gold standard test albeit with some limitations like decrease of sensitivity in low-endemicity areas. 	<i>Schistosoma spp.</i>	Serology	Antibody cross-reactivity with antigens from other helminths.	[107]
			PCR	Discrepancies among study findings regarding test sensitivity as a result of	[108]

	-Indirect methods using clinical, subclinical, or biochemical morbidity markers are not specific.			technical problems.	
ECHINOCOCCOSIS	<ul style="list-style-type: none"> -Considerable phenotypic variability between isolates of <i>Echinococcus granulosus</i> sensu lato -Diagnosis is mostly based on imaging techniques but sometimes they are inconclusive. -The diagnostic sensitivity of the methods can strongly depend on the stage of infection. -Low specificity and sensitivity of the currently available commercial tools. -Prognosis-associated follow-up parameters are still lacking. Invasive procedures following therapeutic interventions in AE patients. -There is a need to develop reliable tools for improved viability assessment. -There is an urgent need for well-validated non-invasive markers. 	<i>Echinococcus granulosus</i>	ELISA	Sensitivity depends on the localization, size, number and stage of cysts. Several other factors could also affect the results of the tests. Cross-reactions with other parasites are common. Antibody persistence.	[109]
			Imaging follow-up	None of the available imaging procedures are currently able to accurately assess <i>E. granulosus</i> viability and/or predict cyst progression/abortion	[110]
		<i>Echinococcus multilocularis</i>	Serology	No single accurate assays for the follow-up: need for combining cytokine and chemokine levels with other circulating markers. Poor correlation between the presence of antibodies in animal serum and worms in the intestine	[110, 111]

Table 3. Microfluidic devices for the diagnosis of malaria infected cells.

Usage	Device/Platform	Highlights	References
Characterization of disease states of single cells	Electric impedance microflow cytometry	Allows differentiation of infected from uninfected RBCs as well as among different <i>P. falciparum</i> intraerythrocytic asexual stages	[119]
	A polydimethylsiloxane microfluidic channel	Potential tool for studying the invasion mechanism as well as performing antimalarial drug assays	[120]
Malaria diagnosis	A cell microarray chip	Offers higher sensitivity in the detection of malaria infected erythrocytes than conventional light microscopy	[121]
	A lab-on-chip	Capable of detecting all <i>Plasmodium sp.</i> with a DL for <i>Plasmodium falciparum</i> of 2 parasites/ μL of blood	[122]
	Polydimethylsiloxane microfluidic channels	Indicate that surface morphologies can serve as an alternative biomarker for malaria diagnosis	[123, 124]
Separation of infected and non-infected cells	Dielectrophoresis based continuous separation	Higher inter-particle distance between red blood cells and plasmodium falciparum infected red blood cells	[125]
Detection of <i>Plasmodium berghei</i> in blood	Acoustic fields to lyse cells	DL of 30 parasites in a microliter-sized blood sample	[126]
Estimation of physical splenic filtration of infected cells	A MCD filtration model	Identify rheological diversity in RBC populations	[127]
Separation of infected cells based on their deformability	A microfluidic device precisely controlling pressure	Potential to study the pathophysiology and the effect of drugs	[128]
	A hyperbolic converging microchannel for continuously monitoring cell deformation in the extensional flow region	Overcomes the limitation of conventional methods by reducing experiment time	[129]
	An automated microfabricated deformability cytometer	Measure mechanical deformability and biomechanical properties of cells. Especially applicable to heterogeneous cell populations	[130]
	A simple long straight channel microfluidic device	An ideal technique for on-site iRBCs enrichment in resource-limited settings	[131]
	A 2-microm microfluidic channel	In contrast to <i>P. falciparum</i> -infected RBCs, mature <i>P. vivax</i> -infected RBCs readily became deformed through 2-microm constrictions	[132]
Measurement of the density of single living cells	A microfluidic mass sensor	Identifying <i>Plasmodium falciparum</i> malaria-infected erythrocytes	[133]
Imaging malaria parasites	A lensfree on-chip microscope	Imaging in thin blood smears	[134]
Monitoring heme dynamics and/or detecting hemozoin in malaria infected cells	A RALS approach	Utility of the technique as a diagnostic and monitoring tool for minute sample volumes	[135]
	A magneto-optical method	Detect parasites at very low densities at the ring stage and in the case of the later stages	[136]

Table 4. List of some successfully applied sensors of parasites reported in the literature.

Parasite	Bio-Sensing Approach	Highlights	Targets and/or Detection Limit	References
<i>Leishmania sp.</i>	Immunosensor-based assay (monoclonal antibody coupled to a bioelectronic device) for detecting antigens quickly	Quantify amastigotes in organs for studies on pathogenesis and immunity	Amastigotes	[142]
<i>Plasmodium sp.</i>	Aptasensor device based on cationic polymers and gold nanoparticles	Allows detection of the two main species of malaria (<i>P. vivax</i> and <i>P. falciparum</i>)	Lactate dehydrogenase	[143]
	Rolling-Circle-Enhance-Enzyme-Activity-Detection system (REAAD)	Detection of malaria parasites in crude blood samples with a colorimetric detection system	Topoisomerase I Activity	[144]
	A label-free DNA biosensor based on quartz crystal microbalance (QCM)	Specific for <i>P. falciparum</i> detection	Merozoite surface protein 2	[145]
	A miniaturized imaging system: sub-pixel resolving optofluidic microscope	Combination of microfluidics and inexpensive image sensors an on-chip device	RBCs infected with <i>P. falciparum</i>	[146]
	A disposable plastic chip and a low-cost, portable, real-time PCR machine	Containing a Peltier element for thermal cycling and a laser/camera setup for amplicon detection.	DL of 2 parasites / μL^{-1} of blood	[122]
	A microfluidic device to measure red blood cell deformability (infected cells) A droplet microfluidics platform	Combination of microfluidic and controlled pressures DL of less than one parasite / μL^{-1} in single drops of unprocessed blood or saline	Deformability values of uninfected and parasitized cells Micrometer-sized products derived from the action of topoisomerase	[128] [137]
<i>Cryptosporidium parvum</i>	A microbead immunoagglutination assay combined with Mie scatter detection in a microfluidic device	DL of ≤ 1 oocyst per large volume of water with the potential to be used in field situations	Cryptosporidium oocyst wall proteins (COWP)	[147]
	A polydiacetylene(PDA) liposome chip based on fluorescence	Non-labeling detection of the waterborne pathogen	DL of 1×10^3 oocysts / mL^{-1}	[148]
	A microfluidic device equipped with a micromesh and FITC-labeled antibody.	Combination of micromesh for entrapment of oocysts with fluorescence immunoassay	High-throughput counting of oocysts	[149]
<i>Schistosoma japonicum</i>	A piezoelectric immunosensor	Mixed self-assembled monolayer membrane (mixed SAM) technology	<i>S. japonicum</i> antibody (SjAb) directly detected in the serum	[150]
	Glutaraldehyde or chitosan cross-linked electrochemical immunosensor	High sensitivity and broad linear range response	<i>S. japonicum</i> antigen ($50 \mu\text{g} / \text{L}^{-1}$ Optimal concentration)	[151]
	A liquid-phase piezoelectric immunosensor (LP-PEIS)	Detect antigens in patients' sera as well as ELISA but in a simple and quick operation	<i>S. japonicum</i> circulating antigen (SjCAg)	[152]

<i>Schistosoma haematobium</i>	On-chip imaging: mini-microscopes constructed from webcams and mobile phone cameras	Low-cost diagnostics of urogenital schistosomiasis.	Fields images of <i>S. haematobium</i> eggs	[153]
<i>Giardia lamblia</i>	A PEMC biosensor immobilized with a monoclonal antibody	The cysts bind to the antibody on the sensor changing the resonant frequency	DL of 1-10 cysts/mL without a preconcentration step	[154]
	Optofluidic microscopy imaging	Successful microscopy imaging by flowing/scanning the target objects across a slanted hole array	Imaging of both <i>Giardia</i> trophozoites and cysts	[155]
<i>Trypanosoma sp.</i>	Gold electrode modified with a thiol sensitized with parasite proteins	100% specificity for the samples studied	Human anti- <i>T. cruzi</i> IgG	[156]
<i>Strongyloides stercoralis</i>	An immunological assay for diagnosing strongyloidiasis based on a novel diffraction-based optical biosensor technology	Serological assay based on real-time optical diffraction (NIE dot)	Antigen called NIE derived from <i>Strongyloides stercoralis</i> L3-stage larvae	[157]
<i>Entamoeba histolytica</i>	Portable screen-printed electrodes for the label-free electrochemical detection	Nano-yeast-scFv probes	<i>E. histolytica</i> cyst antigens	[158]
<i>Babesia bovis</i>	A device based on impedance spectroscopy combined with microfluidic	Sorting based on microfabricated cell cytometer	Allows single cell analysis of normal and <i>B. bovis</i> infected red blood cells	[159]
<i>Lucilia cuprina</i> (Cutaneous myiasis)	Electronic nose technology	Array of sensors that react to volatile chemical compounds	Discriminate infection measuring parasite odour on the day of larval implantation	[160]

PEMC, piezoelectric-excited millimeter-sized cantilever; DL, Detection Limit