wjpmr, 2020,6(6), 276-284

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Review Article

ISSN 2455-3301

WJPMR

CLINICAL BIOTECHNOLOGICAL DETECTION OF SOME PARASITES

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Article Received on 21/04/2020 **Article Revised on 11/05/2020** **Article Accepted on 01/06/2020**

SUMMARY

Molecular testing is increasingly used to supplement or replace conventional microscopy-based methods of parasite identification. Potential benefits of molecular methods such as nucleic acid amplification tests include increased sensitivity, ability to differentiate morphologically similar organisms. A variety of technologies have been applied, and some specific and general pitfalls and limitations have been identified. This review provides an overview of the multitude of methods that have been reported for the detection of blood and intestinal parasites and offers some guidance in applying these methods in the clinical laboratory and in epidemiological studies.

KEYWORDS: Biotechnological detection – Molecular testing –Clinical Diagnosis-Blood Parasites –Intestinal Parasites.

INTRODUCTION

Immunoassays for parasitic antigens are not available for most parasitic infections. Advances in molecular detection by nucleic acid amplification may improve the detection in asymptomatic infections with low parasitic burden. Rapidly accumulating genomic data on parasites allow the design of polymerase chain reaction (PCR) primers directed towards multi-copy gene targets, such as the ribosomal and mitochondrial genes, which further improve the sensitivity. Parasitic cell or its free circulating parasitic DNA can be shed from parasites into blood and excreta which may allow its detection without the whole parasite being present within the portion of clinical sample used for DNA extraction. Multiplex nucleic acid amplification technology allows the simultaneous detection of many parasitic species within a single clinical specimen. In addition to improved sensitivity, nucleic acid amplification with sequencing can help to differentiate different parasitic species at different stages with similar morphology, detect and speciate parasites from fixed histopathological sections and identify anti-parasitic drug resistance.

In the past; parasitological diagnosis was depending on detection of parasitic stages; either by the naked eye or microscopically .Recently, alternative methods were used for improvement of diagnosis of parasitic diseases. These included immunoassays, molecular-based approaches, and proteomics using mass spectrometry platforms technology. So, the detection of parasitic infections depend on several laboratory methods in addition to clinical history symptoms, travel history, and geographic location of patient.

Among the laboratory investigations ; some newer serology-based assays that are highly specific and sensitive are now available ;, such as the Falcon assay screening test ELISA (FAST-ELISA),[1] Dot-ELISA,[2,3] rapid antigen detection system (RDTS)[4] and luciferase immunoprecipitation system (LIPS).[5] Moreover, molecular-based approaches such as loop-mediated isothermal amplification (LAMP).[6] real-time polymerase chain reaction[7] and Luminex[8] showed very high potential in parasitic diagnosis ; with increased specificity and sensitivity. Lastly, proteomic technology is now available for the detection of biomarkers using tissues or biological fluids from the infected host.

Serology sometimes is considered as the only diagnostic tool when biologic samples or tissue specimens are unavailable. Serology-based diagnostic tools can be divided into: antigen-detection assays and antibody-detection assays. These include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), and all its derived tests such as the Falcon assay screening test ELISA (FAST-ELISA) and the dot-ELISA. Also, other assays include the hemagglutination (HA) test, indirect or direct immunofluorescent antibody (IFA or DFA) tests, complement fixation (CF) test, and immunoblotting and rapid diagnostic tests (RDTs).

Concerning diseases caused by some blood parasites; we have Typanosomiasis caused by Trypansoma brucei, *Chagas disease by Trypanosoma cruzi*,Babesiosis by *Babesia microti, Leishmaniasis by Leishmania species,Malaria by Plasmodium species and Toxoplasmosis by Toxoplasma gondii as xamples.*

*As regards some famous intestinal parasitic diseases; we have Cryptosporidium parvum and C. hominis as a causative agent of Cryptosporidiosis, Schistosoma mansoni causing S*chistosomiasis, Fasciola gigantica and F. hepatica for Fascioliasis,Taenia solium for Taeniasis disease / Cysticercosis, *Echinococcus granulosus and E. multilocularis for hydatidosis, Wuchereria bancrofti, Brugia malayi, B. timori and Loa loa for Filariasis as well as Strongyloides stercoralis for* Strongylodiasis .

For Trypanosomiasis; the diagnostic tools include microscopy,[9] ELISA,[10,11] Dot-ELISA or Dipstick,[12] Direct or Indirect hemagglutination assay(DHA or IHA),[13] Direct or indirect immunofluorescence assay(DFA or IFA)m,[14] Polymerase chain reaction(PCR)[9] Real--time polymerase chain reaction(RT-PCR),[15] Loop-mediated isothernal amplification(LAMP)[16] Oligochromatography Polymerase chain reaction (OC-PCR)[17] and Mass Spectrometry (Laser desorption mass spectrometry = LDMS, Matrix-assisted laser desorption/ionization time of flight= MALDI-TOF, Surface-enhanced laser desorption/ionization time of flight =SELDI-TOF).[18,19]

As regards Chagas disease; diagnosis is through microscopy,[20] ELISA,[21–24] Dot-ELISA or Dipstick,[25] Radioimmunoprecipitation assay(RIPA-ELISA),[26,27] Immunoblot,[28,29] Rapid diagnostic test(RDT),[30] PCR.[31–33] and LAMP.[34]

For Babesiosis ;diagnostics include :Microscopy,[35] ELISA,[36] DFA or IFA,[37,38] and PCR.[39,40]

Leishmaniasis is diagnosed by microscopy,[41] Dot-ELISA or Dipstick,[2,42] DHA or IHA,[42] PCR,[43] RT-PCR,[44–46] Quantitative nucleic acid sequenced-based amplification (QT-NASBA),[47] PCR-ELISA,[46,48] and OC-PCR.[49]

Malaria can be detected by microscopy,[50] Falcon assay screening test (FAST-ELISA),[51,52] DFA or IFA,[53] RDT,[54] PCR,[55] RT-PCR.[4,55] QT-NASBA.[56,57] Real-time quantitative nucleic acid sequenced-based amplification (RT-QB-NASBA),[58] LAMP.[59–63] Luminex.[64] PCR-ELISA.[65–67] and Mass Spectrometry (LDMS, MALDI-TOF, SELDI-TOF).[68–70]

Toxoplasmosis can be demonstrated by ELISA,[71] DFA or IFA,[72] Immunoblot,[73] PCR.[74,75] and RT-PCR.[76]

Concerning Cryptosporidiosis ; it can be detected by microscopy,[77] ELISA.[78,79] DFA or IFA[78] PCR,[80] RT-PCR,[81] LAMP[82] and Luminex.[83]

For Schistosomiasis ;microscopy can be used[84] ELISA[85] FAST-ELISA[1] Dot-ELISA or Dipstick[86] Immunoblot,[87] PCR,[88] RT-PCR,[89] and OC-PCR[90]

Fascioliasis can be found through microscopy,[91] ELISA,[91] FAST-ELISA.[92] Dot-ELISA or Dipstick.[93] Immunoblot.[92] and Mass Spectrometry (LDMS, MALDI-TOF, SELDI-TOF).[94]

Regarding Taeniasis disease / Cysticercosis;ELISA demonstrated to be important.[95] Immnoblot,[96] PCR,[97] LAMP[98] and Mass Spectrometry (LDMS, MALDI-TOF, SELDI-TOF).[99]

Concerning hydatid disease ;microscopy is used for diagnosis.[90] ELISA.[102–106] Dot-ELISA or Dipstick.[100] DHA or IHA,[101] Immunoblot.[101]

Filariasis can be detected by Microscopy,[102] ELISA,[103] Dot-ELISA or Dipstick,[104] DHA or IHA,[105] luciferase immunoprecipitation system (LIPS),[106] PCR [107] and PCR-ELISA.[108]

Strongylodiasis is diagnosed by Microscopy,[109] ELISA,[110] DHA or IHA,[111] DFA or IFA,[112] Immunoblot,[113] LIPS.[114] PCR.[115] and RT-PCR.[116]

Due to the type of antigen preparations used (e.g., crude, recombinant purified, adult worm, eggs) and the use of nonstandardized test procedures; the results were variabe. Cross-reaction leading to false-positives and misdiagnosis is also a problem; especially in regions where more than one parasite is endemic. For all these reasons, there is still a need to improve the diagnostic approaches available. Since the advent of the polymerase chain reaction (PCR), parasitologists have turned to molecular-based approaches for improving the existing diagnostic tools. This approach is represented by some tests as nucleic acid based approaches. This represents traditional PCR; including nested and multiplexed PCR. Real-time PCR (RT-PCR) can detect several parasitic infections. Newer technologies such as loop-mediated isothermal amplification and Luminex-based assays are also used as possible new approaches for the diagnosis of parasitic diseases. Moreover, multiplexed PCR helps for the detection of multiple sequences in the same reaction tube proving useful in the diagnosis of several parasitic infections simultaneously.[117]

Real Time polymerase Chain Reaction (RT-PCR) system allows the quantification of the original template's concentration through the use of various fluorescent chemistries, such as Sybergreen, Taqman probes, fluorescence resonance energy transfer (FRET), and Scorpion primers.[7] The concentration is measured through comparison to standard curves. This eliminates the need to visualize the amplicons by gel electrophoresis; thereby greatly reducing the risk of contamination and the introduction of false-positives.

Loop-mediated isothermal amplification (LAMP) is a unique amplification method with extremely high specificity and sensitivity able to discriminate between a single nucleotide difference.[6] It is characterised by the use of six different primers specifically designed to recognise eight distinct regions on a target gene, with amplification only occurring if all primers bind and form a product.[118] Studies demonstrated the rapidity and the improved specificity and sensitivity obtained using the LAMP assay.

Luminex xMAP technology is a bead-based flow-cytometric assay that allows the detection of various targets simultaneously. The microsphere beads can be covalently bound to antigens, antibodies, or oligonucleotides that will serve as probes in the assay. Up to 100 microspheres are available each emitting unique fluorescent signals when excited by laser ; therefore allowing the identification of different targets.[119] Adapted to the study of parasites, the Luminex assay could identify multiple organisms or different genotypes of one particular organism during the same reaction utilizing very low volume. The approach could prove useful in the study of antigenic diversity and drug-resistance alleles and for the diagnosis of parasitic diseases. The high-throughput capability of the Luminex system confers it a clear advantage over the use of labour-intensive microscopy for large scale studies.

We know that proteins are the main catalysts, structural elements, signalling messengers, and molecular machines of biological tissues. So, proteomic studies are able to provide substantial clinical relevance. Accordingly proteins can be utilized as biomarkers for tissues, cell types, developmental stages, and disease states as well as potential targets for drug discovery and interventional approaches. Proceeding further, we think that the next generation of diagnostic tests for infectious diseases will emerge from proteomic studies of serum and other body fluids. Recent advances in this area are as a result of introduction of mass spectrometry platforms capable of screening complex biological fluids for individual protein and peptide "biomarkers." Proteomic strategy can identify proteins in two ways: bottom-up and top-down approaches. In the bottom-up approaches, the proteins in a biological fluid are proteolytically shattered into small fragments that can be easily sequenced and the resultant spectra are compared with those in established peptide databases. This is the protein equivalent of "shotgun" genomics. Bottom-up strategies are difficult to quantitate and cannot identify modified molecules (e.g., alternately spliced, glycosylated). Since each open reading frame in the human genome is thought to generate at least 10 modified proteins, this issue is a major limitation.[120]

The classic top-down strategy is 2-dimensional gel electrophoresis. Top-down strategies seek to identify proteins and peptides (and their natural variants) in complex biological fluids. Proteins are resolved in the first dimension based on pH (a process called isoelectric focusing) and in the second dimension by their molecular weight. This technique is labour intensive, and low throughput and requires large amounts of sample. Other techniques used for the expression analysis of proteins are matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), surface-enhanced laser desorption ionization time of flight mass spectrometry (SELDI-TOF MS), liquid chromatography combined with MS (LC–MS–MS), isotope-coded affinity tags (ICAT), and isotope tags for relative and absolute quantification (iTRAQ). The development of automated, high-throughput proteomic technologies such as MALDI-TOF and SELDI-TOF MS has enabled large numbers of clinical samples to be analyzed simultaneously in a short time. These platforms have made "population-based proteomics" feasible for the first time (reviewed in.[120] All proteomics-based diagnostic efforts seek to identify biomarkers that, alone or in combination, can distinguish between "case" and "control" groups.

The main limitation of SELDI compared to MALDI resides in the fact that SELDI has lower resolution and lower mass accuracy. Moreover, SELDI is unsuitable for high molecular weight proteins (>100 kDa) and is limited to the detection of bound proteins on to the ProteinChip Array.[120]

For parasitic diseases; studies have focused on SELDI. The SELDI, a derivation of MALDI, allows sample binding to chemically active ProteinChip surfaces. Several types of ProteinChip arrays are present with differing abilities to bind proteins with different chemical (anionic, cationic, hydrophobic, metallic, and normal phase) or biological (antibody, enzymes, receptors) properties, thereby allowing the direct analysis of proteins from complex biological samples without the need for prior separation by 2D gel electrophoresis. The output of the SELDI is a spectrum of mass-to-charge ratios (m:z values) with their corresponding relative intensities (approximating to relative abundance).[120]

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