

Chapter 1 : Voller, A. [WorldCat Identities]

Atoms for better health care Immunodiagnosis of parasitic infections Tools are evolving to help control diseases afflicting over million people.

Advanced Search Abstract Background. One major obstacle to schistosomiasis prevention and control is the lack of accurate and sensitive diagnostic approaches, which are essential for planning, targeting, and evaluating disease control efforts. Based on bioinformatics analysis, we identified a multigene family of saposin-like protein SAPLP in the schistosome genomes. Antibodies to the 3 antigens could be detected in the serum samples of rabbits infected with cercariae as early as 3–4 weeks after infection. Although enormous progress has been made in schistosomiasis prevention and control, the condition is still far from being satisfactorily controlled [3]. Recently, an outbreak of schistosomiasis haematobia was detected in France, Germany and Italy, and schistosomiasis has become an emerging threat in Europe [4–6]. One major obstacle to schistosomiasis prevention and control is the lack of accurate and sensitive diagnostic tools, which are essential for planning, targeting, and evaluating schistosomiasis control efforts [7–10]. Currently, the standard method for diagnosing schistosomiasis is the conventional parasitological detection of eggs in stool or urine, such as the World Health Organization’s recommended Kato-Katz method [11]. More and more studies have shown a discrepancy between the prevalence based on conventional parasitological detection and that based on molecular diagnostic methods for schistosomiasis [14–17]. Immunodiagnosis is more sensitive and less time consuming than parasitological diagnosis and has become a more attractive option for the diagnosis of schistosomiasis, especially for atypical forms of schistosomiasis, and for disease surveillance [13]. Serological methods, such as the circumoval precipitin test, the indirect hemagglutination assay, and the enzyme-linked immunosorbent assay ELISA , are used to detect schistosome antigens or specific antibodies against them [18 , 19]. Unfortunately, cross-reactivities with other parasitic diseases presenting in the epidemic areas substantially lower the specificity of these methods that use crude schistosome antigens extracted from eggs or adult worms [8 , 14]. Therefore, the ideal antigens for the immunodiagnosis of schistosomiasis should be pure proteins and should be schistosome specific or have extremely low homology with the antigens of other parasites. Xu et al [20] identified a novel protein SjSP that exhibited high sensitivity and specificity for schistosomiasis japonica diagnosis. They systematically assessed this novel protein in a field study, and the results showed that compared with the Kato-Katz method, a 6-fold increase in sensitivity was achieved using the SjSP protein as a diagnostic antigen. To date, there has been no biological characterization of the SjSP protein because no homologous gene has been found in other organisms. We systematically characterized and analyzed the gene expression profile, localization, and immunological features of the SjSAPLP family. Based on the thorough search, we collected all members of the SAPLP family from the currently available genomic and protein databases. First, sequences were aligned using Clustal X software <http://www.ebi.ac.uk/ClustalW/>. The relative expression level of each gene was analyzed using SDS 1. Primers were designed to amplify the appropriate region of the proteins without the N-terminal putative signal peptides Supplementary Table 2. Serum samples were collected before infection and 1–6 weeks after infection. All patients were confirmed by examining eggs in stool samples with the Kato-Katz method, except in 3 samples that were confirmed using the miracidium hatching test but showed negative results with the Kato-Katz test. All serum samples from humans were obtained with informed written consent, and the protocol was approved by the ethics committee of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences, Beijing. All procedures performed on animals in this study were conducted in accordance with the animal husbandry guidelines of the Chinese Academy of Medical Sciences and with permission from the Experimental Animal Committee with ethical clearance No. All tested serum samples were diluted at 1:10. Optical density values were read at 450 nm by a microplate reader, and all tests were performed in triplicate on each test plate. The worms were imaged using fluorescence microscopy Nikon. Statistical Analysis The data analysis was performed using the VassarStats statistical platform <http://vassarstats.net/>. The cutoff value of the positive test was set at 2.

Voller A, Bidwell DE, Bartlett A, Edwards R. A comparison of isotopic and enzyme-immunoassays for tropical parasitic diseases. *Trans R Soc Trop Med Hyg.*

This is an open access article distributed under the Creative Commons Attribution License , which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Abstract Methods for the diagnosis of infectious diseases have stagnated in the last 20â€”30 years. Few major advances in clinical diagnostic testing have been made since the introduction of PCR, although new technologies are being investigated. Pressing needs include more rapid tests without sacrificing sensitivity, value-added tests, and point-of-care tests for both high- and low-resource settings. In recent years, research has been focused on alternative methods to improve the diagnosis of parasitic diseases. These include immunoassays, molecular-based approaches, and proteomics using mass spectrometry platforms technology. This review summarizes the progress in new approaches in parasite diagnosis and discusses some of the merits and disadvantages of these tests. Introduction Currently, the detection and diagnosis of parasite infections rely on several laboratory methods in addition to clinical symptoms, clinical history, travel history, and geographic location of patient. The primary tests currently used to diagnose many parasitic diseases have changed little since the development of the microscope in the 15th century by Antonie van Leeuwenhoek. Furthermore, most of the current tests cannot distinguish between past, latent, acute, and reactivated infections and are not useful for following response to therapy or for prognosis. Recent developments in new diagnostic tools, however, have opened new avenues for a vast improvement in parasite detection. Secondly, molecular-based approaches such as loop-mediated isothermal amplification LAMP [6], real-time polymerase chain reaction [7], and Luminex [8] have shown a high potential for use in parasite diagnosis with increased specificity and sensitivity. Thirdly, proteomic technology has also been introduced for the discovery of biomarkers using tissues or biological fluids from the infected host. The aim of this review is to highlight the potential for these new technologies in parasite diagnosis. For convenience, old and new parasitic diagnostic tools are summarized in Tables 1 and 2. Diagnostic tools for the detection of specific blood-borne parasitic diseases. Diagnostic tools for the detection of specific intestinal parasitic diseases. Microscopy For many years, microscopy has been the only tool available for the detection of parasites through inspection of blood smears [10 â€” 14], tissue specimens [15 â€” 17], feces, lymph node aspirates [18 , 19], bone marrow [20], and even cerebrospinal fluid [21]. However, sample preparation for direct observation is time-consuming, labour intensive, and proper diagnosis depends on qualified laboratory technicians. In the case of slide reading, a second independent reading is preferable, but not always required for accurate diagnosis. If need be, divided readings are resolved by a third reader. In endemic regions, where resources are limited, this proves to be difficult and misdiagnosis can significantly impact patient care. In reality, all major intestinal helminth infections are still solely dependent on microscopy for diagnosis. As for other parasite infections, many are confirmed by the use of microscopy in conjunction to other methods of diagnosis including serology-based assays and more recently molecular-based assays. Serology-Based Assays In situations where biologic samples or tissue specimens are unavailable, serology alone is the gold standard for diagnosis. Serology-based diagnosis tools can be divided into two categories: Although the ease of use and turnaround times for serologic assays are similar to microscopy, serology-based assays are more sensitive and specific. It becomes important for individuals whose blood smears do not permit identification of the parasite e. Classifying an infected asymptomatic patient as negative could lead to transmission of the parasite during blood transfusions or organ transplants. In the case of Fasciola infection, serology tests have also been shown to be useful in the confirmation of chronic fascioliasis when egg production is low or sporadic []. Finally, having these tests readily available allows for the monitoring of parasite clearance following therapy. In the past, the method has been applied to the study of malaria [32], fasciolosis [], schistosomiasis reviewed in [], and taeniasis []. However, this technique is subjected to the same drawbacks as most serology-based tests. Antibodies raised against a peptide from one parasite protein may cross-react with proteins from other species. Moreover,

antibodies raised against a peptide may react in some assays but not in others and some regions of a peptide may be more immunogenic than others. In the dot-ELISA, the plastic plate is replaced by a nitrocellulose or other paper membrane onto which a small amount of sample volume is applied. The choice of binding matrix greatly improved the specificity and sensitivity of the assay by reducing the binding of nonspecific proteins usually observed when plastic binding matrixes are used. The principle is similar to the immunoblot. The dotted membrane is incubated first with an antigen-specific antibody followed by an enzyme-conjugated anti-antibody. The addition of a precipitable, chromogenic substrate causes the formation of a colored dot on the membrane which can be visually read [2]. The benefits of this technique include its ease of use, its rapidity, and the ease of result interpretation. It is fast, and cost-effective and more importantly can be used in the field e. For all these reasons, the Dot-ELISA has been and still is extensively used in the detection of human and animal parasitic diseases, including amebiasis, babesiosis, fascioliasis, cutaneous and visceral leishmaniasis, cysticercosis, echinococcosis, malaria, schistosomiasis, toxocariasis, toxoplasmosis, trichinosis, and trypanosomiasis all reviewed in [3]. In the last few years, published studies have demonstrated the use of the dot-ELISA for the detection of *Fasciola gigantica* [], *Haemonchus contortus* [], *Theileria equi* [], *Trypanosoma cruzi* [], and *Trypanosoma brucei* [34]. In the latter study the researchers were able to demonstrate that the dot-ELISA had better sensitivity and specificity than the ELISA in the detection of antineurofilament and antigalactocerebrosides antibodies in cerebrospinal fluid of subjects infected with African trypanosomes. They attributed the greater sensitivity and specificity of the dot-ELISA to the use of the nitrocellulose membrane and showed that their assay was successfully reproducible in the field.

Rapid Antigen Detection System RDTs Rapid antigen detection tests RDTs based on immunochromatographic antigen detection have been implemented in many diagnostic laboratories as an adjunct to microscopy for the diagnosis of malaria. RDTs consist of capturing soluble proteins by complexing them with capture antibodies embedded on a nitrocellulose strip. A drop of blood sample is applied to the strip and eluted from the nitrocellulose strip by the addition of a few drops of buffer containing a labeled antibody. The antigen-antibody complex can then be visualized directly from the membrane [4]. Since the appearance of the first RDTs in the s, major improvements have been made to the technique, making the use of RDTs in rural endemic regions feasible. RDTs are useful in the identification of P. In addition, they are useless at detecting very low-density infections. PCR-based approaches remain the tool of choice in that situation. However, as reported by Murray et al. Malaria RDTs have recently been introduced in African countries to help prevent misdiagnosis of malaria infections and to subsequently reduce the practice of presumptive treatment [49]. In fact, the tendency to treat slide-negative samples with antimalarials is still a common phenomenon. Finally, misuse of antimalarials could lead to the appearance of drug-resistant strains. Basically, an antigen of choice is fused to the enzyme reporter *Renilla luciferase Ruc* and expressed as a Ruc-fusion in mammalian cells to allow for mammalian-specific posttranslational modifications. Some of the advantages of the LIPS technology include its rapidity and accuracy in detecting infected patients. Sensitivity is improved in part by the use of mammalian cells which produce fusion antigens free of contaminating bacterial proteins. This greatly facilitates the separation between negative and positive samples. A LIPS assay can be performed in 2. By decreasing the incubation times of a normal LIPS assay, they were able to minimize cross-reaction. Many of the O. Of interest for the application of this technique in the field is the observation that blood obtained by finger-prick contaminated with red blood cells and other components did not interfere with the LIPS assay. As discussed, immunodiagnostic tests have some serious limitations. Parasitic diseases such as amebiasis, cryptosporidiosis, filariasis, giardiasis, malaria, cysticercosis, schistosomiasis, and African trypanosomiasis do not have commercially or FDA approved antibody detection tests for their diagnosis. Experimental results have been too variable due to the type of antigen preparations used e. Cross-reaction leading to false-positives and misdiagnosis is also a problem, especially in regions where more than one parasite is endemic. Despite the fact that some parasites in South America share common epitopes, it is common to see coinfection with *Trypanosoma cruzi* and *Leishmania* species []. It is also a problem in Africa, where cross-reactivity exists between filarial and other helminth antigens []. To a lesser extent but nonetheless important is the inability of antibody-detection tests to differentiate between past and currently active

infections []. Furthermore, antibody-detection tests cannot be used in parasitic infections that do not develop a significant antibody response. This has been observed in some individuals carrying *Echinococcus* cysts [] or during cutaneous leishmaniasis [http:](http://) Similarly, in the case of African trypanosomiasis diagnosis, such tests are of limited use because seroconversion occurs only after the onset of clinical symptoms [83]. For all these reasons, there is still a need to improve on the current diagnosis approaches available. Since the advent of the polymerase chain reaction PCR , parasitologists have turned to molecular-based approaches in the hopes to better the existing diagnosis tools. Nucleic Acid-Based Approaches The many limitations of microscopy and serology-based assays have influenced parasitologists towards the use of gene amplification methods made possible with the advent of the polymerase chain reaction PCR. Newer technologies such as loop-mediated isothermal amplification and Luminex-based assays have also emerged as possible new approaches for the diagnosis of parasitic diseases. Molecular-based approaches based on nucleic acids offer greater sensitivity and specificity over the existing diagnostic tests. Moreover, multiplexed PCR allows for the detection of multiple sequences in the same reaction tube proving useful in the diagnosis of several parasitic infections simultaneously []. 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. When multiplexed, RT-PCR allows for the high-throughput analysis of different sequences in one single-closed tube reaction []. Running the multiplex assay not only reduced the cost per test but also allowed for a rapid turnaround time, the assay taking only three hours to complete. It is a clear advantage over microscopy which is labour intensive and time-consuming with slow turnaround times especially during high-throughput settings. Similarly, multiplexed RT-PCR proved useful in differentiating drug-sensitive strains of malaria []. This is important for proper antimalarial prescription. In another example, Diez et al. This allowed immediate treatment of the patients before reactivation of Chagas disease could occur. These examples demonstrate that efficient and early diagnosis can directly impact patients care and that PCR-based approaches have the potential to help in making the right choice for treatment. Although DNA-based methods have shown excellent sensitivity and specificity, the introduction of these methods in daily laboratory practice is still uncommon especially in rural endemic regions. In addition, as observed with many serology-based assays, PCR-based methods also suffer by the lack of standardization [22]. DNA extraction, choice of primer sets, and use of various amplification protocols are all factors that may cause this diversification in results []. Adding an automated DNA extraction step would certainly improve PCR assays for use in the diagnosis of parasitic diseases. Loop-Mediated Isothermal Amplification LAMP 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 []. Recently, parasitologists have adapted the LAMP approach for the detection of several parasitic diseases including the human parasites *Entamoeba* [], *Trypanosoma* [68], *Taenia* [], *Plasmodium* [70], and *Cryptosporidium* [], the animal parasites *Theileria* [] and *Babesia* [,], and even to the identification of vector mosquitoes carrying *Plasmodium* [73] and *Dirofilaria immitis* [] parasites.

Chapter 3 : parasitic | Definition of parasitic in English by Oxford Dictionaries

The immunodiagnosis of parasitic diseases is briefly reviewed. It is concluded that immunological tests, in conjunction with clinical and other evidence, may give a great deal of help in the establishment of an accurate diagnosis.

The test utilizes cultured merozoites to detect antibodies directed against proteins that are unique to *S.* Antibodies produced to other organisms can be differentiated. Positive CSF indicates that parasites have penetrated the blood-brain barrier and stimulated a local immune response. If the integrity of the blood-brain barrier is compromised, circulating antibodies may leak across and produce a false positive test result. False negative results have been rare, but may occur. Some horses may simply fail to respond to the *S.* The possible causes of false negative responses are important to consider so that affected horses are not misdiagnosed. Horses that initially tested positive have become negative after several weeks of treatment and are apparently recovered. Chronically affected horses may test negative and still be infected or the horse may still exhibit neurologic signs. We speculate that this may be due to permanent CNS damage and that parasites are either no longer present or, antibody production is below test sensitivity. The use of PCR testing aids in parasite detection when antibody level is low. Acute cases that test negative should be re-tested in two to three weeks. However, the incubation period appears to be sufficiently long to allow production of detectable amounts of IgG before the onset of clinical signs in most cases. As was discussed before, one exception with a very short incubation period has been observed. Use of PCR as a diagnostic tool has been developed. The DNA fragments from the *S.* It was therefore suggested that this *S.* Further study was performed to determine the phylogenetic relationship of *S.* This was done based on the sequence of the small ribosomal subunit gene of *S.* This study led to development of species-specific amplification primers which could be utilized for wildlife testing to elucidate the life cycle of *S.* Other research examined the specificity and sensitivity of polymerase chain reaction PCR for detection of *S.* The test was both sensitive and specific, however, it appears to be related to the presence of parasite in the CSF. Therefore, it appears that the PCR testing may be useful prior to development of an antibody response in the CSF or in chronic cases where the antibody level has waned below detectable levels. Other testing modalities have been used to help determine the disease status of neurologic horses. Research at OSU would suggest that this information is of limited value, but may help determine if the CSF sample has been contaminated during the collection process. Other tests that appear to have more value in determining the true disease status of neurologic horses are the CSF indices. These are calculations based on serum and CSF concentrations of albumin and immunoglobulin G. The AQ will help determine the permeability of the blood-brain barrier BBB and helps to determine if the CSF sample collected was contaminated with serum or blood. The IgG Index will determine if the antibodies in the CSF were produced in the central nervous system intrathecal or whether they are due to leakage from the serum as well. Although the bugs have not been worked out conclusively, these tests may have an important role in augmenting clinicians in the diagnosis of EPM.

Chapter 4 : Diagnosis of Parasitic Diseases: Old and New Approaches

Parasitic diseases remain as a major public health problem worldwide, not only based on their historically high morbidity and mortality rates, but also because risk factors associated with their transmission are increasing. Laboratory diagnosis and particularly immunodiagnosis is a basic tool for.

Chapter 5 : Immunodiagnosis of parasitic diseases.

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Chapter 6 : Immunodiagnosis in parasitic disease.

Counter immunoelectrophoresis using cellulose acetate as the supporting medium was used as a rapid screening test for amoebic abscess. All the sera from 40 cases gave positive results. No false.

Chapter 7 : Immunodiagnosis | College of Veterinary Medicine

Clinical Immunology Newsletter Vol. 4, No. 1 January Immunodiagnosis of Parasitic Infections Fausto G. Araujo, DVM, Ph.D. Department of Immunology and Infectious Diseases Research Institute, Palo Alto Medical Foundation Palo Alto, California For many years the diagnosis of several parasitic diseases depended on the direct demonstration of the parasite itself or its cysts, eggs, or.

Chapter 8 : Immunodiagnosis of Parasitic Diseases

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Chapter 9 : Immunodiagnosis of Parasitic Diseases - Europe PMC Article - Europe PMC

This book is excellent and includes some of the best up-to-date reviews currently available on the immunodiagnosis of human helminthic diseases. Some important helminthic infections of humans are usually not directly demonstrable without biopsy or surgery, notably the zoonotic infections causing hydatid disease, cysticercosis, trichinellosis and toxocarasis.