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Review Article

A REVIEW OF CURRENT IMMUNODIAGNOSTIC TECHNIQUES IN PROTOZOOLOGY

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ABSTRACT

Immunodiagnostic techniques are based on the principles of immunochemistry. Current diagnostic methods employed in protozoology include ELISA technique, counter immunoelectrophoresis, immunoblotting technique, immuno-fluorescence Assay methods and complement fixation tests. These methods have been effectively employed in protozoology (exemplified by malaria and Trypanosomiasis). These methods have been found to be effective, sensitive and specific and are useful tools in research and fieldwork especially in manpower-constrained areas of the tropics and sub-tropics.

Key words: *Immunodiagnosis, Protozoology, Techniques.*

INTRODUCTION

The importance of parasitological diseases cannot be over-emphasized. Malaria, for instance constitutes a menace to human development, having been ranked as the world's number one cause of morbidity and mortality, followed closely by schistosomiasis. The need for appropriate diagnosis of parasitic diseases has become important to both the clinician and the parasitologist as well.

The development of immunodiagnostic methods for the appropriate identification of parasites in human specimens be it blood, stool or urine has become a sine qua non to effective clinical medical practice. This has become a great challenge to the parasitologist and the researcher at large. The development of modern immunoassays is based primarily on their increased sensitivity, combined with acceptable specificity[1].

Parasites can be identified at their various developmental stages - the eggs or ova, the larva or the adult. Immunodiagnosis employs the principle or application of antigens resulting from these parasites or the antibody, which the host organism mounts against the parasites.

The invention of the microscope in the early centuries made it possible to identify parasitic eggs or larva in human specimens, making use of their characteristic morphological structures. This however, has its limitations in field studies and surveys. With the advancement in immunology and related fields in human medicine, it has become feasible to isolate parasitic antigens and use these to provoke antibodies in hosts, the presence of which in sera can be used to identify these parasites or their components hence giving rise to the concept of immunodiagnosis.

Parasites can be considered according to their biological groups such as protozoa, helminthes, bacteria or viruses. Immunodiagnostic techniques have been found relevant in the laboratory diagnosis of each and every one of the above-mentioned groups. For the sake of this review, emphasis will be placed on protozoan parasites.

Immunodiagnostic techniques are based on the principles of immuno chemistry, which itself, is a branch of chemistry which deals with the detection of and quantitation of chemical substances by the measurement of antigen-antibody interactions[2]. In these cases, the antibody is used as the reagent to detect the chemical substance (the antigen) of interest. On the other hand, specific antigens can be employed to detect the presence of specific antibodies in the sera of humans.

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Antibodies are immunoglobulins, which are capable of binding to a variety of natural and synthetic antigens including proteins, carbohydrates nucleic acids, lipids and other chemicals. Immunoglobulins (1g) consist of five general classes designated as IgG, IgA, IgM, IgD and IgE. Of all these, IgG is the most prevalent and the most often employed.

The National Committee for Clinical Laboratory Standards (NCCLS), USA[3] define certain characteristics of anti bodies which make them appropriate for agglutination reactions and hence, their application in immunodiagnostic techniques. These include specificity, potency, labeling and stability.

Amongst the five most important parasitic diseases, which have been marked for special attention by the World Health Organization (WHO), three protozoan diseases namely, malaria, leishmaniasis and trypanosomiasis are included. Greater research is encouraged in these diseases considering the fact that their prevalence continues to be high[4]. There is a clear need for techniques that diagnose clinical infections and for use in fieldwork and surveys. Such techniques also are needed to study the epidemiology of such infections i.e. the pattern of transmission in the community, and the detection of persons who have had the infection in the past or carriers.

The method of diagnosis, which uses the immunological binding reaction between antibodies and antigens, is termed immunodiagnosis and the assays for measuring these antibodies or antigens are called immunoassays.

IMMUNO DIAGNOSTIC TECHNIQUES IN AMOEBIASIS/AMOEBIC LIVER ABSCESS

Amoebiasis is the infection caused by a specie of protozoan organism known as Entamoeba histolytica. One of the rare complications of amoebic dysentery is amoebic liver abscess and this occurs when the parasites are carried to the liver via the portal circulation. Immunodiagnostic techniques, which have been employed in the diagnosis of amoebiasis and amoebic liver abscess, are:

(a) Latex slide test

(b) Cellulose Acetate Precipitation (CAP) Test.

The Latex Slide Test: The test detects antibodies to *E. histolytica*. It is also known as the fomouze Bichio-Latex Amibe test. It takes 5 minutes to detect *E. histolytica* and is available in a 25-test kit.

Cellulose Acetate Precipitation (CAP) Test: This test method detects antibodies to *E. histolytica* antigens in serum of any patient with invasive amoebiasis. It is highly specific and useful in epidemiological studies.

IMMUNODIAGNOSTIC TECHNIQUES IN GIARDIASIS

Giardiasis is the infection by specie of protozoan organism known as *Giardia lamblia*. The organism is a flagellated protozoa which exists in two forms: a non-infectious pear shaped trophozoite (9-20mm) inhabiting the small intestine and the highly infectious cyst form which is elliptical in shape and ranges in size from 8-12 mm[5]. The trophozoites are highly labile and die quickly once outside the host body while the cyst form are more resistant and survive for days outside the host body[5]. The parasites easily contaminate water in endemic areas and people travelling to such areas can easily contract the infection[6,7,8,9].

Direct transmission of *G.lamblia* occurs in healthy carriers by food contamination[10, 11]. Stephens and colleagues[12] report that high-risk subjects include young children, immune-compromised people, and those without previous exposure. Phillips and Colleagues[13] have reported that recently, giardiasis has become a common sexually transmitted disease. Animal reservoirs are very important in transmission, and have been implicated in several cases of water contamination[14,8,15].

The most common and traditional method of diagnosis of giardiasis has been stool microscopy. However, this method requires extensive experience and the presence of intact cysts in the stool sample. Immunodiagnostic techniques have been employed in the diagnosis of giardiasis. These methods include:

(a) ELISA
(b) Counter Immunoelectrophoresis
(c) Immunoblotting technique
(d) Direct Immuno Fluorescence Assay (DFA)

ELISA: This means enzyme-linked immunosorbent assay and employs trophozoite immune rabbit serum to detect antigen of *G. lamblia*. The procedure is fairly simple to perform and exhibits increased sensitivity when compared to microscopy. Various techniques[16] are employed to examine faecal samples and accuracy of results is dependent on the skill of the technician. In 50 -70% of faecal examinations, positive results have been obtained[17,18]. Several investigators[19,20,21] have found the ELISA technique to be sensitive, specific and easy to perform.

Janoff and Colleagues[20] compared three different diagnostic techniques including ELISA and found similar results. Comparing counter immuno electrophoresis and ELISA with microscopy, these investigators noted that the methods have identical sensitivity, specificity, positive predictive value and negative predictive value. However, the false positive rate by ELISA was 24% (10 of 42) in day care centres but only 3% (1 of 32) in healthy adults as corroborated by microscopy. The authors concluded that ELISA might be more sensitive than microscopy, which is considered the reference standard, and that result may be dependent, in part, on the epidemiology of infection in the study subjects.

In a similar study as above, Azia and colleagues[1] made similar observations while Garcia and colleagues[22] in their own study found both sensitivity and specificity of the DFA method to be 100% when compared to the conventional microscopy.

Furthermore, Beth and his colleagues[23] obtained positive results with ELISA technique in 92% of samples of giardiasis subjects studied. Two percent (2%) of the subjects showed false positive results. Like the previous investigators mentioned, they also found the ELISA technique to be simple, sensitive and specific for the diagnosis of *Giardia lamblia* infections.

HAEMOFLAGELLATES

These are parasites of great public health importance characterized by the possession of flagellae. Representatives of this group include:

(a) *Trypanosomes* which causes trypanosomiasis

(b) *Leishmania* which is responsible for Leishmaniasis.

The three most important diseases of public health importance to be considered in this review are:

(a) American trypanosomiasis or chagas disease
(b) African trypanosomiasis
(c) Visceral Leishmaniasis or kala-azar.

CHAGAS DISEASE

This highly debilitating illness is caused by species of Trypanosomes known as *Trypanosoma Cruzi*. Four immunodiagnostic techniques have been employed in the diagnosis of American Trypanosomiasis or chaga's disease. These include:

1) Complement Fixation Test (CFT)
2) Indirect Fluorescent Antibody Test (IFAT)
3) Indirect Haemagglutination Test (IHAT)
4) Enzyme Linked Immunosorbent Assay (ELISA)

The complement fixation test (CFT): detects anti T-cruzi antibodies in sera of patients suffering from Chagas' disease and like other techniques, it becomes positive one month following infection and remains so even after treatment. Among the four methods mentioned above, ELISA technique is the most extensively applied and is simpler than others. Breniere and colleagues[24] evaluated the Micro Double Diffusion test (MD) in Bolivian patients with Chaga's disease and found a sensitivity of 84%. The test easily detected T. cruzi antigens in sera of the patients used in the study. It was simple to perform and said to be highly specific. In a similar study, Marco and Colleagues[25] assessed the use of recombinant antigens for the accurate immuno diagnosis of Chagas' disease and obtained better results than when a single antigen was used. In the same study, recombinant ELISA technique was found to be better than conventional ELISA and when it was compared with other techniques like haemagglutination and immunofluorescence, it was found to be the best, producing a specificity and sensitivity of 100%. The workers recommended the use of recombinant ELISA technique in blood transfusion procedures so as to prevent the transmission of Chagas disease. In a related study, Claudia and Rossi[26] evaluated the ELISA technique and compared it with Indirect Immunofluorescent technique (IIF) and found out that ELISA technique exhibited 98.6% sensitivity

and 98.7% specificity respectively as opposed to 94.5% and 96.2% recorded for indirect immunofluorescence. They concluded that the ELISA technique was better than IIF method.

Recently, Gonzalezi and colleagues[27] have concluded a research work where three different protein antigens of T. cruzi have been coupled in polystyrene based latexes and used to develop a novel immunodiagnostic tool for effective diagnosis of Chagas disease. This will soon be put to use in field surveys since it has been found to be simple, very sensitive and specific.

AFRICAN TRYPANOSOMIASIS

This is an acute/chronic protozoan infection caused by two species of trypanosomes called:

(a) *Trypanosoma brucei rhodesiense*
(b) *Trypanosoma brucei gambiense.*

Whereas T. brucei rhodesiense is responsible for the acute infection in man, T. brucei gambiense causes the chronic form of the disease. Immunodiagnostic techniques have been employed extensively in the diagnosis and treatments of Trypanosomiasis. One of the most commonly employed methods is the latex Card Agglutination Trypanosoma Test (CATT). This method is based on the measurement of antitrypanosome 1gM in Cerebrospinal Fluid (CSF). It has been found very useful in the late stages of the disease when treatment is very crucial. One of the handicaps of CATT method is false positive result due to cross-reaction resulting from the presence of IgM in CSF in other conditions such as viral meningitis, tuberculous meningitis and neurosyphilis. However, Lejon and colleagues[28] evaluated the CATT method and found it to be simple, rapid and useful in field studies. It was not found to be useful in T. brucei rhodesiense endemic areas because, it detects only a few of the parasites. The CATT method can be performed using whole blood or diluted serum (to reduce cross reactions) and blood collected on filter paper[29].

VISCERAL LEISHMANIASIS

This is a chronic debilitating condition caused by species of protozoan organisms called *Leishmania donovani*. During the course of the infection, both specific and non-specific antibodies are formed against the Leishmania parasites. Immunodiagnostic techniques have been employed to detect the specific antileishmania antibodies in sera of affected individuals. Three of such methods or techniques include:

(a) Direct Agglutination Test (DAT)
(b) Rapid Latex Agglutination Test (LAT)
(c) ELISA.

These methods have been found to be cheap, reliable and useful in field studies[30].

Cummins and colleagues[31] evaluated the DAT method and compared it to other immunodiagnostic methods. He found out that DAT was more sensitive and specific than the Indirect Fluorescent Antibody Test (IFAT) and the Counter Immunoelectrophoresis (CIE) techniques. The rapid latex agglutination test was also evaluated by Mood and El-Safi[32] and they found the method to be quicker and easier to perform and interpret than DAT. Felix de Lima and colleagues[33] have assessed the use of ELISA methods to diagnose visceral leishmaniasis in lower animals in Brazil. They found the ELISA to be sensitive and specific and suggested its use to detect early infected animals in endemic areas so as to prevent human transmission and spread of the infection.

Furthermore, Krishna and colleagues[34] have also developed a direct ELISA technique using *Leishmania donovani* promastigote antigens for the diagnosis of kala-azar. In the said study, the test was able to differentiate between Kala-azar and other diseases prevalent in Asia and has the potential to be used in developing countries with laboratories that are poorly equipped.

Toxoplasmosis: This is a very deadly disease which is considered a zoonosis. It is due to infection by coccidian parasites known as *Toxoplasma gondi.* The organism naturally infects lower animals like the pig but may incidentally infect man when the later comes into close contacts with affected animals. The most serious form of the disease occurs during pregnancies when the baby in the womb contracts the infection via the placenta leading to congenital toxoplasmosis. Congenital toxoplasmosis is associated with very severe fetal abnormalities.

Immunodiagnostic techniques have been employed

in the diagnosis of toxoplasmosis especially in pregnancy and neonates where the infection is usually severe. Rotmars and colleagues[35] have assessed an indirect ELISA and antibody capture ELISA techniques using a major serological toxoplasma antigen (M.6KD), to detect IgM antibodies in human sera, and also, comparing the result with that obtained by the Immunoblot technique. The investigators concluded that the three methods were highly sensitive but whereas the indirect ELISA gave false positive result, the immunoblot test gave false negative result. The antibody capture ELISA gave no false negative reactions. The specificity was also high.

Two techniques that have been frequently applied in the diagnosis of toxoplasmosis are:

(a) The Sabin Feldman dye test
(b) The Eiken toxoreagent latex agglutination test.

SABIN FELDMAN DYE TEST

This is the most reliable immuno-diagnostic technique in the diagnosis of toxoplasmosis. It is based on complement mediated neutralizing antibody antigen reaction, using live trophozoites of *Toxoplasma gondi* to measure the parasite specific antibody. It is highly sensitive and specific.

EIKEN TOXOREAGENT LATEX AGGLUTINATION TEST

This test is simpler to perform and gives a comparative result to that of the Sabin Feldman test. However, the disadvantage of the latex agglutination test is its difficult interpretation due to the high prevalence of antibodies in most endemic populations as a result of the presence of past and sub clinical infections. Detection of toxoplasma specific IgM is indicative of recent infections. The major limitation of the latex agglutination test is its high cost and the fact that it has to be done in a reference laboratory such as the Centre for Disease Control (CDC) laboratory. Thus, it may not be useful in most field surveys.

HAEMOSPOROZOITES

The most important disease under this category is malaria, which has been ranked number one World parasitic disease. Four species of malaria parasites are important in human infection namely:

(a) *Plasmodium falciparum*
(b) *Plasmodium malariae*
(c) *Plasmodium ovale*
(d) *Plasmodium vivax.*

Amongst all these species, P. falciparum is the most prevalent in the tropics. The diagnosis of P. falciparum malaria has been made easier and simpler by immunodiagnostic techniques. Some of the most recently developed techniques can also detect P. vivax (mixed infections).

Immunodiagnosis of malaria is based on the immunochromatographic detection of two main plasmodium antigens known as Histamine Rich Protein 2 (HRP-2) and the specific Parasite Lactate Dehydrogenase (PLDH). These antigens are produced by the malaria parasite during its developmental cycle in the red blood cells. Extensive studies[36] have been carried out on recombinant and synthetic P. falciparum antigens to assess their immunodiagnostic properties. Six P. falciparum antigens were tested for their immunodiagnostic properties in ELISA and Direct Agglutination Tests (DAT). The authors concluded that the application of molecular P. falciparum antigen to ELISA had the best diagnostic properties. The traditional method of diagnosis of malaria is the identification of the parasites in a blood smear. However, there are cases in endemic areas where blood smear results come out negative whereas, the patient is having serious features or signs and symptoms of clinical malaria. Latonio and colleagues[37] have assessed such cases and found that whereas blood smear gave a false negative report in 22.7% cases, serological test using the 'immunofluorescent Assay (IFA) gave only 1.4% false negative result, thus, emphasizing or highlighting the place of immunodiagnosis in malaria infection. In the study cited above, blood smear and IFA results agreed in 59.74% of the all the cases (where both tests were negative) and disagreed in approximately 5% of results.

In another study, Latonio and colleagues[38] write that malaria simulates other infections such as Salmonellosis (7.46% cases), Leptospirosis (7.46% cases), Respiratory infections (2.98%) and Urinary tract infections (1.49%). This makes immunodiagnosis necessary especially in conditions where something has to be done fast so

as to save life. Indirect Haemagglutination Assays (IHA) have been found reliable in such cases.
Based on the two main malaria antigens; HRP-2 and PlDH, three main blood tests for malaria have been developed.
These are:
(a) HRP 2 test- parasight F.
(b) ICT malaria PF
(c) PLDH Test Optimal.

THE MALARIA ANTIGENS

HRP-2: Is produced primarily by *P. falciparum* and is released into circulation by parasitized red blood cells. The antigen persists in circulation several days after the patient has been fully treated especially in heavy infections. Studies[39] carried out in Mali have shown that about 2-3% of P. falciparum strains found in that country were naturally lacking the gene for the production of HRP-2 antigen (the HRP-2 gene). When these strains of falciparum are involved in infections, the parasight F-test will give a false negative result.

PLDH Antigen: This is a malaria parasite enzyme produced by all species and strains of malaria parasites. It is normally released into the circulation by parasitized ruptured red blood cells. It is also found in urine in small concentrations[40].
Unlike the HRP-2 antigen, PLDH disappears from the circulation once the patient has been successfully treated.

The Para Sight F-Test: This is the first immunodiagnostic test method to be developed. Studies[41, 42,43] carried out in different places in endemic areas to evaluate the test method have shown that the Para Sight F method is easy to perform sensitive and specific for malaria parasite. It is a very useful tool in field surveys.

ICT Malaria PF Test: This test method has been evaluated by several workers[44] and found to be easy to perform, sensitive and specific for P. falciparum infections. The authors showed that the ICT malaria test remained positive seven days after successful treatment of infection due to the persistence of HRP-2 antigen in the blood as noted previously. Recently, ICT combined test has been developed, making it possible to diagnose both falciparum and vivax infections simultaneously.

Optimal Test: This is the most recently developed immunochromatographic rapid malaria strip test available in the market[45]. It is capable of detecting both *P. falciparum* and *P. vivax* (mixed) infections and is useful in differentiation of malaria species due to the fact that there is "antigenic differences between PlDH isoforms[45]. It is easy to perform, sensitive and specific for malaria parasite. Since it is based on PlDH antigen which disappears after treatments, it can be used to monitor responses to drug therapy and to detect drug resistant strains of malaria. The PLDH antigen level in blood reflects the presence of viable malaria parasites in the blood.

CONCLUSION

Traditionally, diagnosis of parasitic infection has been based on microscopic examination for parasites or their eggs in human samples such as urine, blood and stool, or biopsy material. This has been okay for clinical medicine but for quick diagnosis and epidemiology, this approach is viewed critically as practically impossible. This is particularly so in situations when few or no parasites or their eggs are available in specimens for identification, such as in low intensity of infection, in early incubation period, or in late chronic stages of the infection. The development of immunodiagnosis has changed this picture.

Immunodiagnostic techniques have a wide application in parasitology. In certain parasitic diseases, some of these techniques are sensitive, specific and diagnostic whereas in a few others, they have limited value due to non-specificity and cross-reactions. Techniques like the ELISA has made the diagnosis of most parasitic diseases easier, quicker and cheaper and have been applied widely in field studies and surveys where they have proved to be indispensable. For instance, the ParaSight F-test, for malaria parasite can be used to screen so many people for P. falciparum infection in a given population in a short period of time, a task which microscopy alone will not be able to achieve in record time.

Immunodiagnositic techniques are therefore an essential tool for field workers and epidemiologists for effective screening of target populations.
With the recent technological advancement in the Western countries, it is expected that in the near

future, immunodiagnosis will form the bedrock of all laboratory diagnostic methods. These will make diagnosis easier and quicker and enable clinicians to arrive at correct clinical judgements and appropriate treatments of their patients. The World will be better for it.

REFERENCES

1. Azia, Hassan, Beck, Carolyn E, Flux, Mary F., Hudson, M Jame (2001) A comparisms study of different methods used in the detection of Giardia Lamblia Clinical Laboratory Science, Summar 2001. hhtp://findarticles.com/p/articles/mi_3890/is_200107/ai_n8956237/pg-2/.
2. Grigory J. Bulfone. Principles of Immunoclinical Techniques. Biochemistry Vol. 1. (IC) pp. 290-2207
3. The National Committee for Clinical Laboratory Standards (1993). Agglutination analyses. Antibody Characteristics, Methodology, Limitations, and Clinical Validation; Approved Guildline NCCLS document. Vol 13 (15).
4. Castelino J.B. (1986) Immunodiagnosis of Parasitic infections. Tools are evolving to help control diseases affecting over 900 million people. IAEA Bulletin, Summer 1986.
5. Erlandsen, L.S., E.A. Meyer (1984). Giardia and giardiasis: biology, pathogenesis and epidemiology. Plenum Press, New York.
6. Petersen, H. (1972). Giadiasis (Lambliasis) Scand J. Gastroenterology. 7 (Suppl 14): 1-44.
7. Brodsky, R.E., H.C. Spencer, and M.G. Schultz (1974) Giardiasis in American travelers to the Soviet Union. J. Infect. Dis. 130:319-231.
8. Crawn G.F. (1979) Waterburne out break of Giardiasis In Jakubowski, W., J. Hoff (Ed) Water born transmission of giardiasis. U.S. Environmental Protection Washington Pp. 127-1.
9. World Health Organization Scientific Working Group (1980). Parasite related diarrhoeas. Bull. WHO 58:819-830.
10. Pickering L.K., W.E. Woodward, H.L. Duporil, and P. Sullivan (1984). Occurrence of Giardia Lamblia in day care centres J. Ped. 104: 522-526.
11. White, K.E., C.W. Hedberg, L.M Edmonsen, D.B.W. Jones, M.T. Osterholm, and K.L. MacDonald (1989) An outbreak of giardiasis in a nursing home with evidence for multiple modes of transmission. J. Infect. Dis. 160: 298-304.
12. Stevens, D.P., and A. A. Mahmond (1980). Giardiasis: the rediscovery of an ancient pathogen. Curr. Clin. Trop. In Infect. Dis. 1:195-207.
13. Phillips, S.C., D. Mildvan, and D.C. Williams (1981) Sexual transmission of an enteric protozoa ad helminthes in a veneral disease clinic population. New Eng. J. Med. 305: 606.
14. Friend, D.S. (1966) The fine structure of Giardia Muris. J. Cell. Biol. 29:317-332.
15. Faubent, G.M. (1988). Evidence that giardiasis is a zoonosis. Parasit Today 4:66-89.
16. Sun. T. (1980) The diagnosis of giardiasis. Am. J. Surg. Pat. 4:265-271
17. Kamath K, R Murugasu (1974) A comparative study of four methods for detecting Giardia Lamblia in children with diarrhoeal disease and malabsorption. Gastroenterology 66:16-21.
18. Burke J.A. (1977) The Clinical and Laboratory diagnosis of giardiasis. Crit. Rev. Clin. Lab. Sci. 7:373-391.
19. Nash, T.E., D.A. Herrington and M.M. Levinc (1987). Usefulness of an enzyme linked immunoassay for detection of Giardia antigen in faeces. J. Clin. Microbiol. 27:1169-1171
20. Janoff, E.N., J.C. Croft, L.C. Pickering, T. Novotny, M.J. Blaser, C.V. Knisley and L.B. Reller (1989) Diagnosis of Giardia Lamblia infections by detection of parasite specific antigens J. Clin. Microbiol. 27: 431-435.
21. Stibbs, H. (1989). Monoclonal Antibody based enzyme immunoassay for Giardia Lamblia antigen in human stool. J. Clin. Microbiol. 27: 2582 - 2588.
22. Garcia L.S., Brewer T.C., Bruckner D.A. (1979) A comparism of the formalin-ether concentration and trichrome S methods for recovery and identification of intestinal protozoa. Am J. Med. Technol. Nov. 45(11).
23. Beth L.P Ungar, Robert H. Yoken, Theordore E. Nash and Thomas C. Quinn (1984). Enzyme-linked immunosorbent Assays for the detection of Giardian lamblia in faecal specimens. J. Infect Dis Vol 149(1): 90-97.
24. Breniere F.S., Carlier Y, Carrasco R,

Molinedo S, Lemestic J.L. Desjeux, P. Afchain D. (1987). Specific Immunodiagnosis of Chagas disease: Immunodiffusion test using a specific serum anti-Trypanosoma cruzi component 5. Trop Geogr. Med. 39(3): 281-285.

25. Marco A. Krieger, Elza Almeida, Walter Oelemann, Juan J. Lafaille, Josse Borges Pereira, Henirque Krieger et al (1992) Use of Recombinant Antigens for the accurate immunodiagnosis of chagas disease. Am.J. Trop. Med. Hyg. 46(4): 427-434.
26. Clandia D. Partel and Clandio L. Rossi (1998) A rapid quantitative ELISA for immunodiagnosis of Chagas disease. Immunol. Inv. 27(1) 89-96.
27. Gonzalezi Veronica D.G., Valeria S. Garcig Jorge R. Vega, Ivan S. Marcipa, Gregorio R, Merra et al (2010). Immunodiagnosis of Chagas disease: Synthesis of three latex-protein complexes containing different antigens of T. cruzi. Biointerfaces 77(1): 12-17.
28. Lejon et al (1997). Further evaluation of latex IgM assay for IgM quantification in cerebrospinal fluid of sleeping sickness patients. Paper presented 24th ISCTRC meeting. Maputo Maza Mbique 23/9/97 3/10/97.
29. Miezan T. et al (1991). Evaluation of Testryp CAT applied to blood samples in filter paper and to diluted blood in a focus of trypanosomiasis due to Trypanosoma brucer gambiense in Cote d'Ivoire. Bull WHO 69(5): 603-609.
30. Harith A.E. et al (1986) A simple and economically direct agglutination test for serodiagnosis and seroepiemiological studies of visceral leishmaniasis. Transact. Rog. Soc. Trop. Med. Hyg. 80:583-587
31. Cummins A.J. et al (1994) Development of a rapid Latex agglutination for the detection of visceral Leishmaniasis: Trans. Roy.Soc.Trop.Med. Hyg. 88: 300
33. Felix de Lima Valeria Maical, Luciane Biazzono, Ana Clandia Silva, Ana Paula Ferveira Lopes Correa and Maria Cecilia Rui Luvizotto (2005).
34. Krishna Mukerji, Abhijit Pal, Kshudiram Naskar, Dillip K, Ghosi et al (2005). Direct ELISA: A simple immunoassay using L. donovani promastigote for diagnosis of kala-azar. J. Clin. Lab. Analysis 5(4): 299-301.
35. Rotmans, J., W. Duchenne, C. Linscooten, N. Int'l Veld, and A Polderman (2005) Comparative study of three immunoassays for detection of immunoglobulin M. antibodies against toxoplasma gondic. J. Clin. Microbiol. Infect. Dis pp. 535 538.
36. Knobloch M, Schreiber, S. Grokho Sky, A. Scherf (1987). Specific and non-specific Immunodiagnostic properties of recombinant and synthetic P. falciparum Antigens: Eu. J. Clin. Microbiol. 547-551.
38. Latonio A. Arcilla, D; Josefina C. Alzaga, and Valorie F. Chan (1983) The value of immunodiagnosis in problematic tropical and infectious diseases encountered in the Philippines. Phil. J. Microbiol. Infect. Dis 12(2): 147-154.
39. Traore I. et al (1998). Field studies of parasight F test in a malaria endemic area: cost feasibility, sensitivity specificity, predictive value and detection of the hrp-2 gene among wide type plasmodium falciparum in Mal. Am. J. Trop. Med. Hyg (in press 1998).
41. Mbarakurwa, S; Manyame,B; and Shiff, C.J.(1997) Trial of ParaSight F test for malaria diagnosis in the primary health care system Zimbabwe. Trop. Med. Int. Health Vol. 2(6) 544-50.
42. Craig, M.H; and Sharp, B.L (1997) Comparative Evaluation of four Techniques for the Diagnosis of Plasmodium falciparum infections. Trans. Roy.Soc.Trop. Med. Hyg. 91:279-282.
43. Udechukwu, C.U and Onyali I.O (2010) Comparative analysis of different diagnostic techniques for plasmodiasis. Nig. J. Parasitol.31 (2): 108-112.
44. Singh N, Valecha, V.and Sharma, V.P (1997) Malaria diagnosis by field workers using an immuno chromatographic test. Trans. Roy. Soc. Trop. Med. Hyg. 91 pp 396-7.
45. Markler M.T. (1997). Introducing OptiMAL rapid malaria test. Flow Inc 6127 SW orbett. Portland O.R. 97201 U.S.A.