Immunodiagnosis of parasitic infections
Tools are evolving to help control diseases afflicting over 900 million people

by John B. Castelino

Parasitic infections are one of the world's major causes of human illness and suffering. Manifestations of disease range from the fevers of malaria to physical deformities, such as "river blindness" and elephantiasis, resulting from infections by certain filarial worms. Parasitic diseases lower the productivity of the human work force, and some of the apathy encountered in regions where these diseases are endemic may be directly traced to such infections. In the extreme, death results, either as a direct consequence of the parasitic infection or from viral, bacterial, nutritional or other diseases to which the body, weakened by the ravages of parasitic infections, succumbs.

There is a growing acceptance of the importance of health and quality of life in socio-economic development. Hence, increased international interest has been focused on research in the diseases of the tropics, notably by the Special Programme on Research and Training in Tropical Diseases of the United Nations Development Programme (UNDP), the World Bank, and the World Health Organization (WHO). Five of the six diseases selected for attention in the Special Programme are caused by parasites — filariasis, leishmaniasis, malaria, schistosomiasis, and trypanosomiasis.

Despite this focus, the prevalence of parasitic infections appears to remain high. Some 250 million persons have filariasis; a similar number, schistosomiasis; some 400 million people live in malarial regions, with 50 million cases of malaria each year resulting in about one million deaths. The extension of development projects — such as irrigation and resettlement schemes — and the growing density of human populations in the tropics can be expected to further worsen the situation unless effective measures to control parasitic infections are implemented.

So that control measures may be implemented, there is a clear need for techniques that diagnose clinical infections. Such techniques also are needed to study the epidemiology of the infection, i.e., the pattern of transmission in the community, and the detection of persons who have had the infection in the past or who are carrying the infection with few, if any, clinical signs of disease.

Classically, diagnosis has been based on microscopic examination for parasites or their eggs in stool, urine, blood, or biopsy material. But for both diagnosis and epidemiology this approach is viewed increasingly as
impractical. This is particularly so when few, if any, parasites or their eggs are available in body fluids or stool for examination, such as in low intensity of infection, in the early incubation period, in the late chronic stages of the infection, or in infections such as those caused by filarial worms in which the parasites may be present in body fluids only at certain times of the day.

Tools for diagnosis

One of the body's defenses against parasites is its immunological system. On or in parasites, and in their secretory or excretory products, are many proteins. Against some of these (antigens), the body is capable of generating other complex protein molecules (antibodies) that neutralize the antigens by binding to them. Techniques that measure these antibodies or antigens provide alternative tools for diagnosis. This 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, immunoassays.

During the 1960s, there was a recognition of the role of immunoassays in the study of parasitic infections. It was seen that they had a place not only in the research laboratory but also in the field for immunodiagnosis and epidemiology. The most commonly used techniques were based on the reaction between antibodies and antigens that resulted in a visible agglutinated or precipitated product. These techniques were found to be useful, but in some cases they lacked sensitivity. More recently, immunoassays that use reagents labelled with radionuclides, enzymes, or fluorescent substances have become popular because of their potentially high sensitivity in the detection of antigens and antibodies.

Radioimmunoassays (RIAs) and related procedures could provide alternative diagnostic means to the classical parasitological tests. The new assays should be cheap, rapid, technically simple, and well adapted to the demands of large-scale epidemiological surveys, particularly for evaluating the effects of the various national and international schemes of control.

The need for immunological tests is particularly great in assessing the effectiveness of chemotherapy and in detecting infections in which few parasites are available for microscopic examination. The available tests for antibody detection appear to be poor in this respect. However, measurement of antibodies has the advantage of providing indications of the immune response of the infected host. In those infections where specific antibodies are clearly involved in immune defense, as in malaria and schistosomiasis, immunoassays for antibodies can allow identification of protective responses. This may be an important clue to the strategy for research for immunological control of parasitic infections.

Measurement of parasite antigens is a recent alternative to antibody detection, and several research groups are developing assay systems, often based on labelled antibodies. It now appears feasible to measure levels of antigens released by parasites in host serum and urine, and through collaborative programmes the IAEA has helped in the evaluation of such tests.

With the availability of enzyme and fluorochrome tracers, consideration needs to be given to the choice of tracers. Radioisotope tracers provide easy quantitation of the antibody or antigen being measured. Moreover, because of the low non-specific signal in radioactivity measurements, radioisotope tracers are valuable particularly in the developmental stages of antigen detection assays. Once the assay has been established, non-isotopic labels such as enzymes are equally suitable for routine use. Indeed, such tracers offer advantages in many circumstances with the result that radioisotopes may play a diminishing role in the field.

Nevertheless, radioisotope tracers will not disappear. The cost of sophisticated equipment for measuring non-isotopic tracers is not lower than that of radioactivity counters, and radioisotope counting equipment is already widely available. The expertise developed by the Agency over the years in immunoassays using radioisotopes is immediately relevant to other tracers, and it will continue to play a role not only in developing sensitive assays but also in transferring the technology to less advanced Member States.

Radioimmunoassay: Important role

The basic immunoradiometric principles were established some 30 years ago in studies on binding of radiolabelled insulin to antibodies to insulin (anti-insulin antibodies).* The basis for the RIA was laid by the observation that labelled insulin can be displaced by unlabelled insulin in the assay. The competition between the labelled and unlabelled insulin to bind to anti-insulin antibodies (the outcome of which is predictably dependent on the relative concentrations of the different reagents) led to the evolution of competitive binding assays. These have been used in a variety of situations for quantitation of substances present in trace quantities in body fluids.

Immunoassays using an immunological reagent (antibody or parasite antigen) and a radionuclide, an enzyme, or a fluorochrome, have a potential to play an important role in immunodiagnosis and epidemiology. Their sensitivity is high, they use minute amounts of parasite antigens or patient sera, they are applicable to large-scale surveys, and they have the ability to yield quantitative results.

Objectives in Immunodiagnosis

The objective of research in immunodiagnosis of parasitic infections is to develop rapid, cheap, and technically easy tests that can be used in epidemiological surveys to evaluate the effects of various national or international schemes of control in areas where parasitic infections are endemic. It should provide tests that have

a high degree of sensitivity and that are specific for each infection, thus enabling their employment in immunodiagnosis even when few parasites are available for direct parasitological examinations. This is an important consideration in epidemiological surveys since it is recognized that in endemic areas only a portion of the people carrying an infection may present clinical symptoms.

Research in immunoassays also needs to provide tools that assess the effectiveness of chemotherapy or other curative measures, and thereby permit monitoring of treatment. Finally, it should provide tests that identify those individuals who develop immunity to the infection. Such tests will be valuable in assessing the efficacy of vaccine programmes that can be expected in the future when anti-parasite vaccination becomes available.

**New developments encouraged**

Progress in the development of RIAs and related procedures has not yet, however, been so extensive as to replace conventional techniques. The main pitfalls have been the lack of commercial pressure to develop test kits, and the lack of good reagents. The specificity and sensitivity of the immunoassay depends on the technology and on the reagents used. Limitations are now set by the lack of well-defined reagents and the false positive and false negative reactions that are seen as a consequence of this.

The importance of the need for improved immunodiagnostic tests to use in individual or epidemiological studies is widely recognized and reflected in the priorities established by the Special Programme on Research and Training in Tropical Diseases of the UNDP, World Bank, and WHO, as well as by the IAEA’s sub-programme component on parasitic diseases. Recent advances in separation techniques, and the production of antigens and antibodies using genetic engineering and biotechnology, augur well for development of improved serodiagnostic immunoassays using radionuclide and other tracers. This development is being encouraged by both WHO and IAEA.

**Choosing antigens for immunoassays**

Any immunoassay of anti-parasite antibodies will depend on the quality of the antigen. A suitable antigen should be one that reacts with antibodies present in all infected patients. It should be unique for the parasite species, so that it does not react with antibodies produced in infections by other species of parasites. Parasites have a highly complex collection of antigens, many of which are similar to those in other parasite species. The unique antigens need to be isolated from these. Furthermore, the antigen should be free of host substances. This can be a major issue with parasites that are intimately associated with host tissues, such as the malaria parasite.

The antigen also should be obtained in sufficient quantities for large-scale surveys and at a reasonable cost. When it is considered that about 1 gram of *Schistosoma mansoni* worms may enable a preparation of 1 to
Immunoassay systems

Immunoassay systems seeking to measure antigens, antibodies, and circulating antibody-antigen complexes can be basically classified into two groups:

- The competitive binding assays, which depend on the measurement of a substance in a biological sample by allowing it to compete with a similar but labelled substance for reaction with a limited quantity of a binding reagent.
- The non-competitive binding assay, in which the substance to be measured (antigen or antibody) is reacted with a large quantity of a binding reagent (antibody or antigen). The binding reagent is either directly or indirectly labelled with a tracer.

The reagents may be in the liquid phase, and react to form insoluble complexes that can be separated by precipitation. On the other hand, the binding reagent may be adsorbed to a solid support, such as plastic, with the reaction taking place on this surface. Such assays are called "solid phase assays". An assay in which the binding reagent, adsorbed to a solid surface, is reacted with the substance to be measured, and the sequestered substance is then reacted with additional binding reagent, is often called a "solid phase sandwich assay".

Two illustrative examples are shown here.

![Diagram of competitive and non-competitive immunoassays](image)

3 milligrams of purified specific antigen, it can be seen that the analytic method must be sensitive enough to detect minute amounts (nanogram per test) of pure antigens. Such sensitivity is provided by RIAs and related procedures.

New strategies for analysis of mixtures of antigenic material and for demonstration and isolation of immunologically important antigens have evolved with the development of biochemical and genetic engineering techniques. Many employ radionuclide tracers to identify antigens of interest. The Agency’s programme on development of vaccines against schistosomiasis encourages research into preparation of such antigens from the schistosome parasite.

Monoclonal antibodies

In response to a particular antigen, the body normally produces a great variety of antibodies. An important recent development in biotechnology has made it possible to make preparations in which the individual component antibodies are segregated. When all the antibody molecules in a preparation are identical, they are said to be "monoclonal antibodies" or, for short, "monoclons". They react in an identical manner with a single antigen or a part of an antigen.

For the demonstration of parasite antigens, monoclonal antibodies are generally used in a "solid phase sandwich assay". In this procedure the monoclonal antibody, adsorbed to a solid support, is reacted with patient serum and the relevant antigen sequestered from it. The sandwich is completed by reacting this bound antigen with the same or a different monoclonal antibody that is labelled with a radionuclide or other tracers and that will also bind to the antigen.

Assays for antibodies

Both competitive and non-competitive methods have been employed for measuring anti-parasite antibody. Immunoassays for antibodies can diagnose infection and demonstrate immunity, especially when purified antigens are used for the assay. It is generally accepted, however, that while immunoassays for antibodies can reflect present or past infection, they cannot readily distinguish between the two because of the persistence of antibodies in treated patients. This prevents their use in assessing the effectiveness of chemotherapy or other curative measures. Moreover, since there is a lag between the time of infection and the time at which antibodies appear in the circulation, immunoassays for antibodies are often unable to diagnose the infection during the early stages.

These shortcomings have prompted the development of alternative immunoassays for detection of circulating parasite antigens, which are presently being investigated for a number of parasitic infections.

Immunoassays of circulating parasite antigens

Parasite antigens are released into the host’s body fluids by infective parasites and theoretically could provide markers of active infection, as their levels are expected to reflect the intensity of the infection. However, most circulating antigens elicit, in the patient, antibodies that bind to the antigens and remove them from the circulation. These bound antigens are degraded and excreted in the urine. Some parasite antigens may be inaccessible to immunodiagnosis owing to their rapid removal and degradation. Other antigens may be present at very low levels in the circulation so that their measurement needs very sensitive assays based on RIA and related procedures.

A further complication in the assays is the presence of antibodies in the circulation, some of which are free and others that are wholly or partially bound to circulating antigens in antigen-antibody complexes. There will be competition between the antibody reagent of the test and the patient’s antibodies. Furthermore, the presence of other non-specific substances commonly found in blood during parasitic infections are likely to interfere in the assays. Moreover, some circulating parasite antigens may be unsuitable for immunodiagnosis because they are not unique to a single species of the parasite.
The Agency's programme on immunodiagnosis is focused on three parasitic infections — malaria, filariasis, and schistosomiasis. Schistosomiasis can be difficult to diagnose because the symptoms are often confused with those for other diseases. Moreover, the worms causing the infection may not produce the larvae whose presence in the circulation forms the basis for diagnosis by classical microscopic examination of a specimen of the patient's blood. Similar problems, although less acute, may also be presented during low chronic infections with malaria and schistosome parasites or following incomplete cure of such infections.

The Agency's programme encourages the development of immunoassay methods that effectively detect and measure parasite antigens in blood, serum, and urine. It provides a framework for collaborative research through which monoclonal antibodies produced against specific parasite antigens are screened for immunodiagnostic potential using serum and urine from infected and uninfected persons in endemic areas. Those monoclonal antibodies found to be suitable are then used in immunodiagnostic assays and related procedures to assess the applicability of the systems in endemic areas. Encouraging results are being obtained through this programme.

**Schistosomiasis**

In schistosomiasis, a number of monoclonal antibodies with immunodiagnostic potential have been produced by participants in the programme: the University of Leiden, Netherlands; the Institut Pasteur at Lille, France; and the Walter and Eliza Hall Institute Medical Research at Melbourne, Australia. Some of these antibodies have been employed in sandwich assays where one of these has been shown to have a good analytical evaluation, one of these has been shown to have a good potential for large-scale field application. The assay can be expected to replace the classical microscopic examination of salivary glands of freshly killed mosquitoes for assessing the presence of malaria sporozoites. Since the sandwich assay is more rapid and can be performed on dry specimens of mosquitoes that have been stored for several months, it allows the identification of the malaria species and provides objective quantitative estimates of the sporozoite load. The persistence of malaria, especially in Africa, despite decades of implementing control strategies based on insecticides, reinforces the need for such an assay system that can be expected to develop a better understanding of the dynamics and epidemiology of malaria.

In malaria, several monoclonal antibodies have been prepared that detect filarial antigens in patient sera. As in schistosomiasis, the accuracy of the test is better in urine samples since no overlap is found in infected patients and in endemic controls. In malaria, several monoclonal antibodies have been prepared against specific parasite antigens. The antibody was prepared at the New York University Medical Centre in the USA, and is used in a sandwich assay. In preliminary evaluation in Gambia, Burkina Faso, and Mali, the assay shows a good potential for large-scale field application. The assay can be expected to replace the classical microscopic examination of salivary glands of freshly killed mosquitoes for assessing the presence of malaria sporozoites, since the sandwich assay is more rapid and can be performed on dry specimens of mosquitoes that have been stored for several months. It allows the identification of the malaria species and provides objective quantitative estimates of the sporozoite load. The persistence of malaria, especially in Africa, despite decades of implementing control strategies based on insecticides, reinforces the need for such an assay system that can be expected to develop a better understanding of the dynamics and epidemiology of malaria.

Finally, the Agency has a programme that encourages the application of radiation techniques to immobilize anti-parasite antibodies onto solid supports for use in immunoassays. Anti-schistosome antibodies provided by the Pasteur Institute, Lille, France, have been successfully immobilized onto polymer supports by the Japan Atomic Energy Research Institute at Takasaki, Japan, and the Centre for Bio-engineering at the University of Washington, USA. The immobilized antibody is stable at ambient temperatures and retains much of its immunodiagnostic potency.

**“Dip sticks” for the field**

Epidemiological surveys involving collection of blood and urine for immunodiagnosis of infections are often carried out in areas far from a laboratory. The survey team spends several days collecting the samples before returning with these to a central laboratory for processing. This delay presents the problem of keeping the samples under refrigeration to avoid their deterioration. The Agency's programme seeks to develop "dip sticks" for those infections such as schistosomiasis, in which the unique parasite antigen present in the urine or serum is heat stable. The dip sticks would be plastic strips with a stable antibody polymerized at one end that could be reacted with the urine or blood in the field to sequester the antigen. The strip would then be dried and returned to a central laboratory for the immunoassay using enzyme, fluorochrome, or radionuclide tracers.