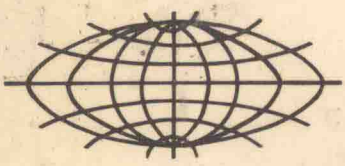
DEVELOPMENTS IN

BIOLOGICAL STANDARDIZATION

DIAGNOSTICS AND VACCINES FOR PARASITIC DISEASES



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PROCEEDINGS OF THE
Joint ESACT/IABS Meeting
on the

DIAGNOSTICS AND VACCINES FOR PARASITIC DISEASES

Proceedings of a Symposium
organized by the
International Association of Biological Standardization
and the World Health Organization
held at
the Royal Academy of Science, Stockholm, Sweden

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21 figures and 17 tables

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WELCOME ADDRESS

Professor Hennessen, Members of the Scientific Committee, Ladies and Gentlemen,

It is a great privilege for me to welcome the distinguished participants to this Symposium on Parasitological Diagnostics and Vaccines, which is being co-sponsored by the International Association of Biological Standardization and the World Health Organization. We are particularly happy that the sponsors chose Stockholm as the venue of this meeting in spite of the arctic temperature.

Parasitic diseases pose great problems of global importance. Compared with investigations in the field of bacterial or viral infections, parasitic diseases offer a special challenge because of their complexity. This holds true from clinical diagnosis to laboratory verification and development of chemotherapeutic or prophylactic measures, in humans as well as in the veterinary field.

Malaria and Schistosomiasis are examples of parasitic diseases which are debilitating hundreds of millions and are killing many individuals. There is therefore a great need and urgency for efforts to diagnose and combat parasitic diseases.

This Symposium is appropriately timed to turn our attention to problems of standardization of the new methods used, to the new reagents and to the candidate vaccines. However, the problem in diagnosis of parasitic diseases is only partly standardization of the emerging new methods. So far, the major problem is identification and availability of useful reagents derived from complex organisms. The preparation of monoclonal antibodies plays an important role for each specific diagnosis. Methods using DNA-probes, new fluorescence techniques, ELISA, and so on, which we will be hearing from the distinguished participants of the Symposium, are adding further diagnostic tools to this field.

There is a need for standardizing efforts in the growing field of commercially available reagents and kits for immunodiagnosis of parasitic diseases.

Steps have also been taken to identify, isolate and prepare or synthesize peptides for possible use as vaccines. It is obvious that techniques using recombinant DNA technology offer possibilities of preparing the required reagents on a large scale.

It is striking how quickly advances in immunology and molecular biology have been applied within parasitology and how these advances have stimulated research within the field.

May I congratulate the Sponsors and the Scientific Committee for adopting such a constructive programme for this Symposium. I wish you every success and many scientific advances during the meeting.

Lars Olof Kallings, M.D.,
Professor, Director, The National Bacteriological Laboratory, Stockholm

OPENING ADDRESS

On behalf of the INTERNATIONAL ASSOCIATION OF BIOLOGICAL STANDARDIZATION I express our gratitude to you, Professor Kallings and Professor Ganelius, for your friendly welcome and your encouraging words.

I can assure you that the WORLD HEALTH ORGANIZATION too is grateful that you are our hosts for the first IABS meeting in this field which will attempt to elucidate what kind of standardization procedures are possible for DIAGNOSTICS AND VACCINES OF PARASITIC DISEASES.

I do not want to conceal that we have been somewhat sceptical when we were first informed of the plans to organize this symposium. Now I am impressed to see that we have participants from all five continents coming from 20 countries. This, indeed, we can take as an indication that the subject our organizers have chosen was well accepted by those working in the field.

We saw the programme that we shall not be confronted exclusively with the highest technology available in industrialized countries, where parasites play no role, but instead, we shall hear and discuss what is and what will be possible in those parts of the world, where parasitic diseases present major health problems.

The fight against disease in the tropics seems to have been somewhat neglected compared with the campaigns against infectious diseases in moderate climates. Eradication of smallpox by WHO proves, however, what success may be achieved in any climate when the weapons for such a fight are available. It is encouraging to see a number of sessions in our programme devoted to such weapons, the vaccines. Progress here seems quite fast, because only a few years ago, immunization against parasites appeared inconceivable.

We all are especially grateful to our organizers, of whom I am glad to mention Gunnel Huldt, Inger Ljungström and Mandayam Tiru. Their incessant endeavour opened a new and promising field for the activities of the IABS. I am sure our appreciation to them could best be expressed by a success of this meeting based on active participation by all of us.

With my hope for such a success I declare this Symposium open.

Walter Hennessen
Scientific Secretary of IABS

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Rapporteur: M. Tiru (S)

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Develop. biol. Standard., Vol. 62, p. 1 (S. Karger, Basel, 1985)

SESSION I

IMMUNOASSAYS FOR PARASITIC DISEASES

Chairman: A. Voller (U.K.)
Rapporteur: Ö. Ouchterlony (Sweden)

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SERODIAGNOSIS OF TROPICAL PARASITIC DISEASES WITH SPECIAL REFERENCE TO THE STANDARDIZATION OF LABELLED REAGENT TESTS

A. Voller

ABSTRACT

The use of immunoassays for tropical parasitic diseases is reviewed followed by a survey of the available test methods and notes on their standardization.

INTRODUCTION

For the purpose of this discussion parasitic diseases will be considered as those with a protozoan or helminthic causative agent. Insofar as the human parasitic diseases are concerned virtually all of them are only of major importance in the tropics and sub-tropics. Because of this distribution, their major impact is on the poorer areas of the world. It is partly for this reason that there has been less commercial activity on immunodiagnosis of parasitic diseases than for instance, on viral diseases, which are widespread in the more developed areas of the world.

One result of this is that most tests and reagents for parasitic diseases are «home-made», usually as a result of interest by an individual or a laboratory in a particular disease. This activity is to be applauded and encouraged but we cannot realistically expect it to be carried on as a continuing service (with adequate quality control) especially when the investigator develops new interests. In general the countries where the parasitic diseases occur have little in the way of immunodiagnostic facilities. The end result of these factors considered above is that the only way in which most parasitic tests can be provided on a continuing basis is for the supra-national agencies (e.g. FAO, WHO) to take on the responsibility. To date there are some examples where this has been successful but there are many others where their enthusiasm has waned once the excitement of the original research has passed.

THE APPLICATIONS OF PARASITIC DISEASE IMMUNOASSAYS

In many tropical environments parasitic diseases are endemic and may affect a majority of the population (e.g. malaria). In such situations serology based on antibody detection has little or no diagnostic value for disease in an individual.

However, it may still be of value in indicating changes in transmission or prevalence, particularly following control measures.

Other diseases which affect a smaller proportion of the population (e.g. Chagas disease) can be usefully diagnosed by antibody assay even in an endemic area.

A further use for serology can be in the confirmation of the effectiveness of therapy following treatment of the disease (e.g. schistosomiasis). However, the rather slow changes in the serological indices minimize the value of this application.

All the above discussion is based on antibody detection. There is now rapidly growing interest in tests for antigen. The interest is that at least some of the classical parasitological procedures based on morphological identification of parasites in tissues or body fluids might be replaced by immunoassays (or by DNA hybridization probes) for the parasites, or their antigens. Progress has already been made along this path and usable tests have been described for amoebiasis and giardiasis, and there are many efforts being made towards malaria diagnosis by these means.

The tests needed for the diseases in the tropical endemic environment discussed above need to be cheap and simple and suitable for mass use if they are to have any impact on the diseases in question.

In contrast there is another rapidly expanding group which consists of visitors (e.g. tourists, consultants, etc.) to the tropics. This has resulted in the widespread presentation of exotic diseases in clinics in non-endemic areas throughout the world. It is here that serology can play a most important role. The presence of specific antibody can often confirm a clinical diagnosis (e.g. trypanosomiasis) and its absence can be used to rule out infection.

Immunotests for this group of people are not subject to the same constraints as those used in endemic areas. Usually the tests will be carried out individually or in small batches, the laboratories are well equipped and staffed, and cost is not an over-riding factor.

THE IMMUNODIAGNOSTIC TESTS

The test procedures most commonly adopted are (1) agglutination methods, (2) gel-based diffusion methods and (3) labelled reagent methods.

Agglutination Methods

These have the great virtue of simplicity and they may be presented as direct agglutination systems or as passive agglutination systems (e.g. passive haemagglutination, bentonite flocculation, latex agglutination). All of these methods are semi-quantitative and are usually based on serial dilutions of patients sera which are used to establish an end-point. Standardization of these tests is difficult — especially the reproducible sensitization of the coated particles for the passive tests. Many of these tests are very susceptible to interference from non-specific factors (e.g. rheumatoid factor, heterophile antibody).

Gel-based methods

Both double diffusion, and immunoelectrophoresis are used on a small scale. These tests are not particularly suited to tropical use and have low sensitivity but the high specificity of immunoelectrophoresis is seen as an advantage particularly for helminthic disease diagnosis.

Labelled reagent methods

These methods are in many cases the immuno tests of choice for parasitic diseases. The usual method for antibody detection is given below.

Reference Antigen on a solid phase + sample (ab) + labelled anti species globulin.

The label on the indicator reagent is usually a fluorescent marker (in immunofluorescence) or an enzyme (in enzyme-linked immunosorbent assay, ELISA).

The test procedures consist of incubation of the test sample (or dilutions of it) with solid phase coated antigen followed by the labelled antiglobulin (and then substrates in the case of ELISA).

Indirect immunofluorescence has been the laboratory reference test for parasitic antibody for many years. It is fairly quick (at least for a few test samples) and it is sensitive and permits use of crude antigen or whole parasites. Disadvantages are the necessity for a fluorescence microscope and the need for skilled staff to interpret complex staining patterns. Although this means that the test is subjective; it can be used with some accuracy.

The enzyme immunoassay (ELISA) usually utilizes soluble antigens adsorbed on to plastic surfaces (or cellulose acetate strips in DOT-ELISA). These soluble antigens are disadvantageous since the extracts are rarely well characterized and vary from batch to batch. The ELISA can be carried out on a single serum dilution and results are more quantitative since they are objectively read in a photometer. In most respects ELISA is comparable with immunoradiometric assay in terms of precision and high sensitivity. The ELISA can be read visually but then it is more subjective and more closely parallels immunofluorescence.

The ELISA test is suitable for other mass use particularly in the microplate format or for individual tests when beads, dipsticks or strips of microwells are better.

Isotopic labels can also be used for parasitic immunodiagnosis either in the classical type of radioimmunoassay (based on competitive binding of labelled reagent with the analyte or in the sample) or, as is more usual now, in sandwich type or immunoradiometric assays (cf. ELISA, IFA). However, the use of isotopic immunoassays is unsuitable for most practical diagnosis because of the short life of reagents and the expensive equipment, but it is possible that the luminescent and delayed fluorescent labels may play a part later on in this field.

STANDARDIZATION

Most parasitic immunoassays are characterized by an almost complete lack of any form of standardization, partly due to reasons enumerated above. However

if results are to be accurate, reliable and reproducible, a certain amount of standardization is imperative. This is even more important in the labelled reagent tests where the potential precision and sensitivity are rarely attained in the parasitic tests. The following section deals with some approaches to the better standardization of parasitic labelled reagent immunoreagents.

Reference sera

(1) Positive panel within laboratory

Any laboratory developing or using parasitic immunoassays must have reference sera from patients proven to have the disease in question. It may be necessary to have a representative panel of sera covering the disease states (e.g. acute and chronic Chagas disease), and diseases caused by types of the parasites which may be morphologically distinct (e.g. malaria infections) or which can only be distinguished by laboratory means (e.g. leishmaniasis).

A smaller number of bigger volume reference positive sera are needed, stored in large number of aliquots, for the purpose of validating test performance over periods of time.

(2) Negative sera within lab

Each laboratory developing test procedures must have panels of sera representing appropriate populations not infected with the disease in question. Again, these should be representative. It is of little value to use healthy adult blood donors from non-endemic areas as controls for pediatric or geriatric populations in endemic areas where other diseases may also be very prevalent. In practice it is very difficult to obtain good relevant control negative sera for widespread diseases (e.g. malaria).

Any new tests also need to be assessed against panels of sera from particular disease conditions (especially auto-immune diseases) which may give rise to factors affecting test performance.

Large volumes of a few well defined «negative» sera will be needed by the diagnostic laboratory to monitor test performance.

(3) External serum banks

Because of the cosmopolitan nature of these diseases there is a requirement for representative serum panels to permit comparison of test results between laboratories within and between countries. WHO has played a major role here in assembling serum banks for schistosomiasis and Chagas disease.

(4) Defined or standard sera

For many diseases there are well defined samples (e.g. toxoplasmosis) denoted either as National or International Standards. These can play a part in assisting laboratories in establishing secondary standards for within-house use. The assignment of units to such materials can be convenient in that it permits ready comparison of results between different tests, and between laboratories and at

different times. There is a real danger, however, in assuming that units of activity in standard sera correspond to mass units of antibody. It is readily apparent that different serum samples may differ in affinity, avidity as well as in specificity. In practice this makes it virtually impossible to define antibody content in polyclonal sera in absolute units.

Antigens

It is the preparation and definition of antigens that pose the most problems for parasitic immunoassays.

Even now most antigens used in the parasitic immunodiagnostic tests are crude extracts. Occasionally these are chemically defined (at least to the extent of protein composition) but this tells us little of the potency in the test systems.

The quantitative reactivity of candidate antigens with panels of sera in the actual test system is the best guide to potency and spectrum of their reactivity. Comparison of this with previously approved antigens gives us the only means of standardization of crude antigens.

The newer methods of antigenic fractionation at least permit the measurement of certain antigen fractions on the basis of physical characteristics (e.g. weight). This allows better comparison between different batches and different preparative procedures but even so final validation of potency in test systems is desirable. It is often necessary for economic reasons to make a compromise on antigen purification e.g. a highly purified and better defined antigen (e.g. CF6 for schistosomiasis) may be unacceptable for reasons of cost and a less purified but much cheaper antigen preparation (e.g. SEA for schistosomiasis) may have to be used.

Monoclonal antibodies have a part to play in measuring the content of particular antigens in mixtures and for affinity purification of such antigens. Once purified such antigens can then be produced by recombinant DNA methods.

Test Methods

All the labelled reagent tests are multi-component systems, and alteration of any one component can drastically affect the outcome of the test. The major variables are as follows:

(1) Solid phase

The plastic solid phase carrier has a major influence on the uptake of antigen and of non-specific components in all the solid phase ELISA and RIA tests. Candidate plastics should be subjected to pilot tests using dilutions of the appropriate antigen, reference positive and negative antisera and conjugate. Those conditions giving maximal reactivity with positive and minimal reaction with negative samples should be used. Different suppliers and batches of plastics and different methods of antigen preparation necessitate revalidation.

(2) Test samples

In this review attention has been directed towards antibody determination in sera. The dilution and diluent used will affect the end result. Serial dilutions can be employed to yield end point titres but most ELISA tests are done on single dilutions of test sera. This dilution must be chosen to yield low values with negative samples, preferably below the visible level for visually read tests. Reference positive samples should at the appropriate dilution give a good easily recognized result in ELISA or IFA (and be readable by the photometer in the case of ELISA) within the accurate part of its reading scale.

Serum diluents often need additives (e.g. wetting agents and proteins such as bovine serum albumin or ovalbumin) in order that non-specific reactions with the solid phase be minimized.

Conjugates

The labelled immuno-reagent is normally an anti-species immunoglobulin which may be labelled with a fluorophore (e.g. fluorescein) or an enzyme (e.g. peroxidase or horse-radish peroxidase). This reagent is clearly a source of much variability. The antiserum can vary both in terms of potency and specificity either of which will affect the outcome of the test. It is preferable now to use monoclonals with defined reactivity e.g. anti-human IgG or anti-human IgM which are γ or μ chain specific respectively. Such monoclonals will usually yield lower background than polyclonal antisera and of course are more consistent. The method of purification of the antiserum i.e. salt precipitation, Protein A, affinity chromatography will all affect its reactivity in the assay systems as will the method of conjugation. Some attempts have been made to provide reference conjugates and a fluorescein labelled anti-human immunoglobulin has been produced and characterized.

Validation

Any new test should be compared with an existing approved test and evaluated on the panels of sera mentioned above. If possible a collaborative trial should be set up to identify problems which the original researcher may have overlooked. It should then be tried in parallel with existing approved tests in large scale field trials before being finally accepted.

CONCLUSIONS

The scope of this review has necessarily been limited and attention has been focussed on antibody detection systems. These clearly have a future role in parasitic immunoassay especially the labelled reagent types. It is highly probable that in the near future there will be greater emphasis on antigen systems and these might replace the traditional parasitological techniques.

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