

PRESENT ASPECTS OF IMMUNODIAGNOSIS OF SCHISTOSOMIASIS

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Facilitated and improved by advances in molecular biology, techniques for the immunodiagnosis of schistosomiasis, including assays based on the detection of antigens circulating in the serum and/or excreted in the urine, have now reached the stage of multi-centre trials. There is a need to complement parasitological techniques as some national programmes are becoming increasingly successful in establishing control of the disease and the classical approach frequently fails to reveal low-intensity infection. Epidemiological survey teams in some areas have tentatively started to use serology and their experience indicates that antibody detection suffices in eradicated or controlled areas with low expected prevalence but that detection of circulating antigens is needed for assessment of the incidence of infection or reinfection in areas recently brought under control.

Before reagents and procedures can be recommended for routine use of national control programmes, the assays must be standardized with sera from clinically well-characterized patients in geographically defined regions, hence emphasizing the need for a reference serum bank. Implementation of serological testing, carried out by national public health laboratories using standardized testing systems, would permit valid comparisons between different areas providing support for decisions regarding national health policies.

Key words: schistosomiasis – immunodiagnosis

Immunodiagnostic techniques for schistosomiasis have been available since early this century (Fujinami & Nakamura, 1909; Taliaferro et al., 1928), but are still not applied in control programmes. This dichotomy is due partly to technical factors and partly to the complex relationship between host and parasite which precludes straightforward interpretation of serological results. In the absence of the complete immunity commonly experienced in bacterial or viral diseases, reinfection is more the rule in parasitic infections, direct demonstration of the causative agent being the only reliable means of diagnosis. Stool and urine examinations are now well standardized and have proved valuable in epidemiological surveys but since the samples investigated normally contain less than 2% of the daily egg output (Bradley 1965), this approach fails in a relatively large proportion of lightly infected individuals (Teesdale et al., 1985; Peters & Kazura, 1987). Furthermore, the day-to-day variation of egg excretion adds to the risk of missing light infections (Teesdale & Amin, 1976; Polderman et al., 1985), a problem brought into sharp focus by the generally lower worm burdens following the introduction of widespread chemotherapy.

The high sensitivity of immunological methods encourages their use for diagnosis. In addition, depending on whether antigens or specific antibodies are utilized as the catching reagent of the assay, serology is capable of addressing direct, as well as indirect, diagnostic aspects. In schistosomiasis, this versatility can be utilized by adapting it for demonstration of, on the one hand, anti-schistosome antibodies and, on the other, antigens released from the several stages of the parasite residing in the blood vessels of the host.

Polyclonal antibodies are generally inadequate for the demonstration of circulating schistosome antigens, due to their minute concentrations in the blood, a problem which could not be tackled before the advent of the hybridoma technique (Köhler & Milstein, 1975). Although monoclonal antibodies (MAbs) are exclusively directed against one epitope only, cross-reactions may occur as epitopes are not unique but can be shared between different microorganisms. The production of MAbs, exclusively reacting with one antigen, is actually more problematic than generating specific antigens by the more complex recombinant DNA techniques. This is due to the fact that the

selected antigen epitope is exactly specified by its DNA sequence, whilst antibody reagents have to be chosen from an array of MAbs with different and, in principle, unknown specificities. Although the growing variety of anti-schistosome MAbs is overcoming this hurdle, the lack of field-applicable techniques continues to limit the routine use of serology in national control programmes.

The different vantage points of laboratory-based researchers and field epidemiologists offers an explanation why the former generally insist on serology, whilst parasitological tests prevail in field practice. However, as laboratory scientists are increasingly working side-by-side with their colleagues in the field a mutual understanding of the various aspects of diagnosis has emerged.

THE TECHNOLOGY

A wealth of different technological approaches has been used in the serologic diagnosis of schistosomiasis. The majority of these concern antibody determination but since circulating antigens can be detected by substituting antigens for catching antibodies, both applications are technically equivalent. A complete inventory of available techniques falls outside the scope of this presentation but the most commonly and widely used methods are briefly outlined. For detailed descriptions reference is made to reviews by Kagan & Pellegrino (1961), Sadun (1967, 1976), Voller

& de Savigny (1981), Maddison (1986) and de Jonge (1990).

Due to low sensitivity and specificity intradermal tests, which are mainly based on immediate type hypersensitivity, have largely fallen out of favour. Although the skin test is not strictly serological, it is included in the schematic overview of generally employed tests (Table) since it relies on immune reactions and its critical characteristics almost completely overlap with those commonly expressed as desirable in the field. Serological assays include various forms of radioimmunoassays (RIA) and the enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence (IF), complement fixation (CF), indirect agglutination of erythrocytes or latex particles and gel precipitation including the circumoval precipitin test (COPT), all of which have their own specific draw-backs. For example, RIA and ELISA depend on complicated equipment and labile reagents; a complex microscope must be used for IF tests; CF requires good laboratory conditions and delicate reagents; gel precipitation techniques usually demand over-night diffusion and are not quantitative. Agglutination techniques, on the other hand, hold some promise as they require neither expensive equipment nor skilled personnel but, for economical reasons, need to include many samples in each round of testing. If need be, however, all these assays can be used with as little as five microlitres of plasma drawn from the top of a haematocrit tube after centrifugation.

TABLE

Tests used in immunodiagnosis of schistosomiasis

Test use	Type	Reagent	Equipm.	Time	Cost	Field
CF	Batch	Delicate	Simple	Medium	Medium	No
DIP STICK ^a	Single	Simple	Simple	Short	Low	Yes
Dot-ELISA	Single	Delicate	Simple	Medium	Medium	Yes
ELISA	Batch	Delicate	Complex	Medium	Medium	No
ID/COPT	Single	Simple	Simple	Long	Low	No
ITH	Single	Simple	Simple	Short	Medium	Yes
IHA/ILA	Batch	Simple	Simple	Medium	Low	No
IF	Single	Delicate	Complex	Medium	High	No
RIA	Batch	Delicate	Complex	Medium	High	No

CF: Complement Fixation
 ID: Immunodiffusion
 IF: Immunofluorescence
 RIA: Radioimmunoassay
 a: Hypothetical assay

COPT: Circumoval Precipitin Test
 ELISA: Enzyme-linked Immunosorbent Assay
 IHT: Indirect Haemagglutination
 ILA: Indirect Latex Agglutination
 ITH: Immediate Type Hypersensitivity

Although the standardized routine of laboratory-based techniques makes bulk processing of sera more reliable, the need for costly equipment and labile reagents cannot often be met. In addition, the frequent lack of infrastructure in the endemic areas prevents the regular transportation of patients or specimens to the laboratory highlighting the need for greater emphasis being placed on developing technologies for field use. Dot-ELISA (Pappas et al., 1983; Boctor et al., 1987; Xue et al., 1987), a technique combining the sophistication of laboratory methods with the simplicity needed in the field, represents progress in this direction. Finally, a dipstick for detection of several different antigens in blood should be mentioned (Snowden & Hommel, 1991). Although this immunoassay, based on sandwich ELISA, dot blotting and antibody-bound colloidal dye particles, has not been tried with schistosome antigens there are no reasons to doubt the feasibility of this interesting approach in this field.

ANTIBODY DETECTION

Determination of specific antibodies may be used diagnostically only in special situations such as, for example, in people originating in non-endemic areas. It can also be used for estimates of prevalence in not previously treated populations but continued antibody production after cure makes this approach impractical for monitoring chemotherapy. On the other hand, a wider field of application may be heralded by the discovery of antigens which preferentially elicit antibodies of short-term duration, e.g. CEF6 (Dunne et al., 1988) and KLH (Mansour et al., 1989). The KLH antigen is of interest not only due to its association with the acute stage but also because a reliable antigen source is secured by the relevant epitope being shared with keyhole limpet hemocyanin (Grzych et al., 1987). In spite of its disadvantages, antibody detection has been tentatively used in well-controlled areas in different parts of the world. In addition, when used with reagents capable of indicating the class and subclass of antibodies, this approach constitutes a powerful research tool for studying the role of isotypes in experimental and natural infection (Feldmeier et al., 1983; Jassim et al., 1987a; Catty et al., 1989; Wilkins et al., 1991; Hagan et al., 1991).

The antigens utilized in serological assays have generally been crude preparations such

as the Soluble Egg Antigen (SEA) and Soluble Worm Antigen Preparation (SWAP) but they also include purified preparations such as the three Major Serologic Antigens (MSA), particularly the 137 kiloDalton (kDa) MSA₁ (Pelley et al., 1977), and the Adult Microsomal Antigens, e.g. MAMA (Hancock & Tsang, 1986). An account of commonly used antigens can be found in reviews by Smithers & Doenhoff (1982), Mitchell & Cruise (1986), and Cesari (1990). A cloned 37 kDa *Schistosoma mansoni* larval antigen has recently attracted interest due to its purported use as a marker for susceptibility. Subjects with apparent high resistance to schistosome infection reportedly exhibit significantly increased levels of antibodies against this antigen compared to susceptible ones (Goudot-Crozel et al., 1989). However, the 31/32 kDa gut-associated antigens of *S. mansoni* (Klinkert et al., 1988, 1989) constitute the only serious effort to produce cloned antigens for large-scale serological surveys. A field trial using an assay based on these antigens, now accepted to be haemoglobulinase and cathepsin B, respectively, showed excellent agreement with positive stool examinations but as many as 75% of egg-negative subjects also tested positively (Ruppel et al., 1990), indicating that further study is required. In a different approach, the catalytic properties of certain schistosome enzymes have been utilized for demonstrating anti-enzymatic reactivity in infected subjects. By treating patient antibodies, bound to a plastic surface by protein A, with crude adult worm antigen and subsequently visualizing the reaction through the addition of various substrates, Cesari et al. (1992) showed that the host immune system recognizes a number of schistosome enzymes.

Three WHO-coordinated collaborative studies have been carried out with the objective of identifying suitable immunodiagnostic test systems for control programmes (Mott & Dixon, 1982; Mott et al., 1987; Yu et al., 1992). The first two studies reported that crude *S. japonicum* SEA is relatively specific and results even correlated to some degree with the intensity of infection. The third study, carried out in China with the aim of comparing different antigens and techniques in local use, corroborated this conclusion and, in spite of the many different techniques used, found a generally good correspondence between results from different laboratories and regions. Although no specific antigen/assay system has

reached the stage of large-scale field trials, the current results suggest that they can be standardized and ultimately used to assess infection on a national level, both qualitatively and quantitatively.

ANTIGEN DETECTION

Adequate differentiation between past and current infection requires assays which target freely circulating parasite antigens. The existence of such antigens was first reported by Okabe & Tanaka (1958, 1961) and later by Berggren & Weller (1967) but, although immunoelectrophoresis improved the sensitivity more than 10-fold (Madwar & Voller, 1977), the required level could not be consistently achieved until MAbs were used for antigen capture (Verwaerde et al., 1979). Although Deelder et al. (1978), using hamsters, reported that one worm pair is capable of sustaining a serum level of 500 nanogram per animal of the circulating anodic antigen (CAA), other antigens seem to be less abundant. Today, however, sensitivity is not a constraint as methodology capable of operating in the picogram/ml range has been developed (Deelder et al., 1989a; de Jonge, 1989a). A list of techniques and their lower detection limits, in relation to the most common antigens, has been published by de Jonge (1992).

A number of circulating antigens have been described and a complete inventory has recently been published by Cesari (1990). The currently most thoroughly investigated antigen of this group is CAA, a 70 kDa antigen originally described by Berggren & Weller (1967), and characterized as a gut-associated proteoglycan (GASP) by Nash (1974). The circulating cathodic antigen (CCA) which was originally named the M-antigen by Carlier et al. (1975) is another, predominantly polysaccharide, antigen emanating from the worm gut (Deelder et al., 1980). The presence of these two antigens in schistosomiasis patients corresponds well with actual infection (Feldmeier et al., 1986; Deelder et al., 1989b; de Jonge, 1990, 1992). A number of other potentially important diagnostic antigens including the phenol-sulphoric acid positive antigen (PSAP) described by Nash et al. (1974, 1977) and a range of schistosome-specific enzymes (Cesari et al., 1987, 1990, 1992) have also been demonstrated in the serum of the host. Antigens have been studied mainly in *S. mansoni* but are generally sufficiently similar in the differ-

ent species to be useful as genus-specific reagents. Although assays based on several of the latter preparations have been developed and used in many laboratories, enough experience to permit large-scale human trials with antigens other than CAA and CCA has yet to be accumulated.

DETECTION OF ANTIGENS EXCRETED FROM THE HOST

Some antigens, depending on size and configuration, are cleared by the kidneys and the first demonstration of excreted schistosome antigens in the host was actually made in the urine of *S. japonicum*-infected patients (Okabe & Tanaka, 1958, 1961). Since then, urinary analysis has produced evidence of antigens in schistosomiasis due to *S. haematobium* (Sherif, 1962) and due to *S. mansoni* (Shoeb et al., 1968). However, the interest in this approach could not be sustained until specific MAbs became available, bringing sensitivity and specificity into line with required levels. Although recent research has been devoted to detecting CCA in the serum (Feldmeier et al., 1986; Deelder et al., 1989b), it had in fact earlier been demonstrated in different excretions from the host including breast milk and urine (Carlier et al., 1975, 1978). Deelder et al. (1980) found a molecular weight heterogeneity of CCA varying from 50 kDa to over 300 kDa, whilst others have reported sizes confined to the 40-45 kDa range (Carlier, 1978; Kestens, 1988a). It was previously thought that CAA could not pass the renal filtration barrier but its molecular weight has now been shown to be 70 kDa (Kestens, 1988b) which is near the exclusion limit. Reexamining urine samples from schistosomiasis patients using a highly sensitive ELISA, de Jonge et al. (1989b) were finally able to demonstrate the excretion of this antigen. Another, excreted schistosome polysaccharide was found in different populations in Cameroon using a mouse MAb of the IgM class (Ripert et al., 1988, 1989, 1990). Positive test results correlated well with egg output in individuals with schistosomiasis due to *S. mansoni*, *S. haematobium* as well as *S. intercalatum*. Finally, ecdysteroid schistosome hormones have been demonstrated in the urine of schistosomiasis patients (Nirde et al., 1982; Koolman & Moeller, 1986) but data from animal experiments do not seem to confirm the utility of this approach (Baswaid et al., 1989). Regardless of antigen, it is clear that sample collection does not have to be invasive

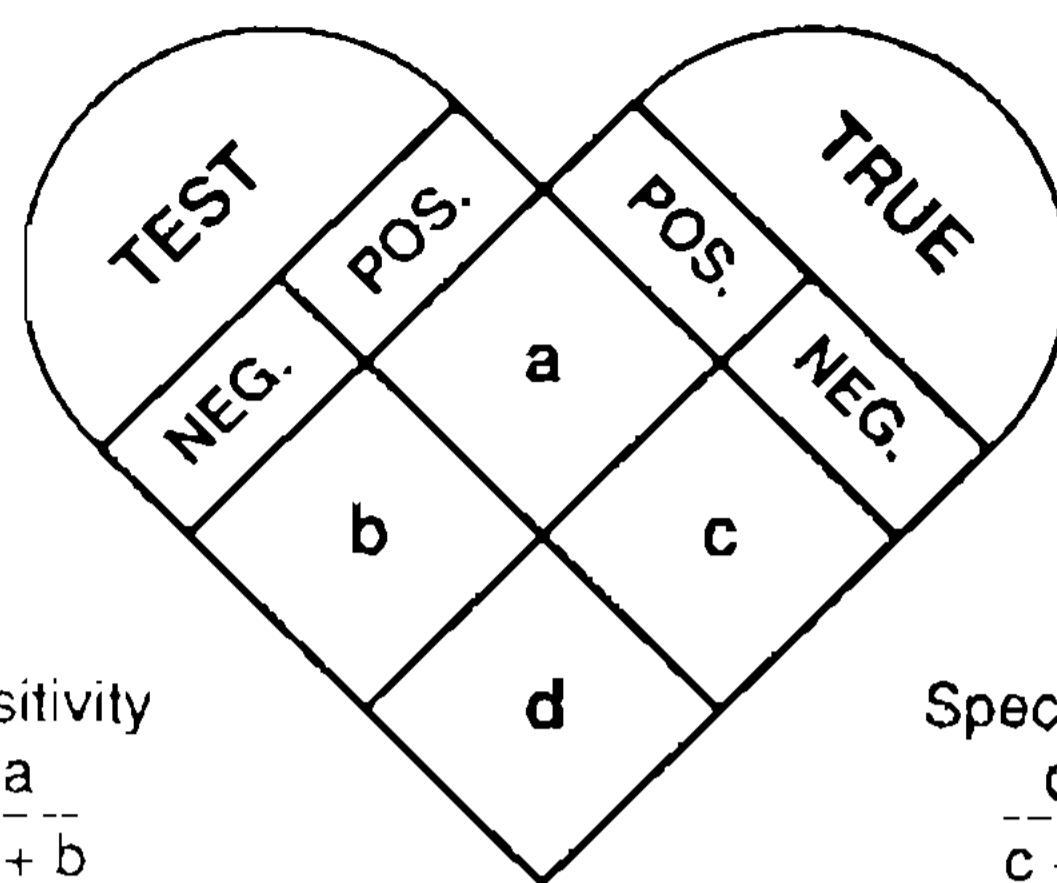
but although a focus on excretions rather than serum will probably have a high rate of acceptance, more experience with excreted antigens is needed before the technology can be properly evaluated.

THE ROLE OF IMMUNE COMPLEXES

The continuous release of schistosome antigens into the blood of the host promotes the formation of aggregated antibody and antigen. Indeed, immune complexes have been shown to form deposits in the glomerular basement membrane, the only pathology unrelated to egg production, (Mahmoud & Woodruff, 1975; Andrade & van Marck, 1984). The presence of immune complexes in serum has been reported in patients infected with *S. mansoni*, *S. haematobium* and *S. intercalatum* by Bout et al. (1975), d'Amelio et al. (1981) and Zwingenberger et al. (1987), respectively. A positive correlation with egg output (Santoro et al., 1980) and with severity of disease (Galvão-Castro et al., 1980) has also been reported. All immunoglobulins, except IgD, and most subclasses have been demonstrated in such immune complexes (Feldmeier et al., 1983, Jassim et al., 1987b) and both CAA and CCA have been described in glomerular deposits in patients with active *S. mansoni* infection (Sobh et al., 1988). Quantification of immune complexes in serum is, however, a relatively complicated procedure involving precipitation with polyethylene glycol, cleavage by acidification followed by immunoelectrophoresis (Bout et al., 1977). However, a novel method, claiming easy and rapid simultaneous determination of free and complex-bound antibody to human immunodeficiency virus (HIV)-1 (Thomas et al., 1990), could have a bearing on this issue.

TECHNICAL VALIDATION

Before implementation of an assay in practice, its characteristics must be appraised in terms of sensitivity and specificity. Insufficient attention to this has produced a disproportionate growth in the number of serological techniques leaving basic methodological parameters inadequately addressed. In addition, as pointed out by Maddison (1986), the relation between prevalence and test results is often ignored leading to a failure to account for how accurately an assay performs under any given conditions. Since this bears an important general significance for serological surveys it deserves a detailed description.



$$\text{Sensitivity} = \frac{a}{a + b}$$

$$\text{Specificity} = \frac{d}{c + d}$$

$$\text{Predictive positive value} = \frac{a}{(a + c)}$$

$$\text{Predictive negative value} = \frac{d}{(b + d)}$$

$$\text{Test efficiency} = \frac{(a + d)}{(a + b + c + d)}$$

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Fig. 1: the four diagnostic categories and their interrelations.

ASSAY PERFORMANCE IN RELATION TO PREVALENCE

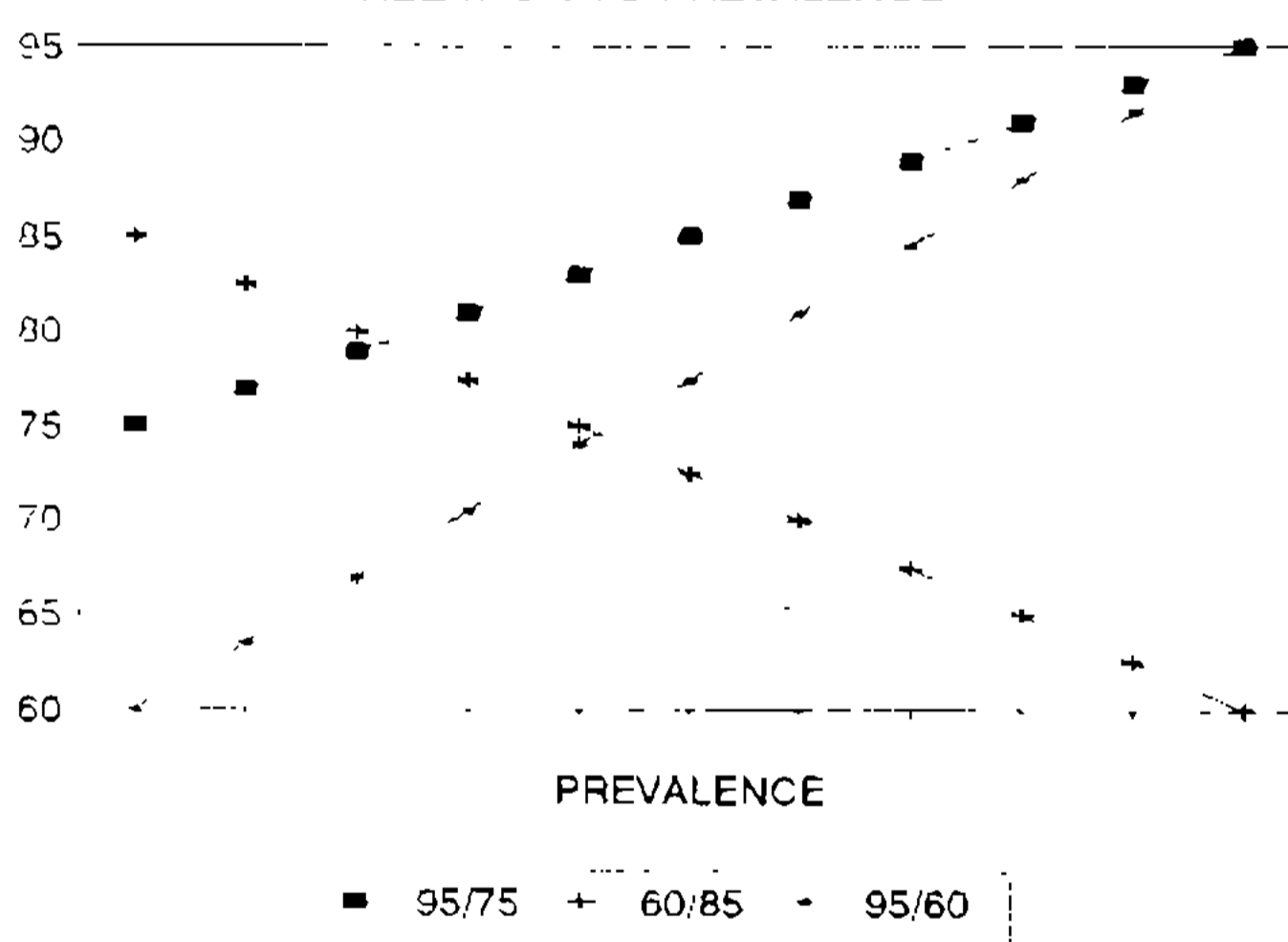


Fig. 2: the overall reliability of test results at different rates of prevalence using three different assays with the hypothetical sensitivity to specificity ratios of 95/75, 60/85 and 96/60, respectively.

The relation of test results to the real situation can be divided into four possible categories (Fig. 1). These groups may be used for calculating test sensitivity which gives the degree of absence of falsely negative reactions and test specificity which corresponds to the degree of absence of falsely positive reactions. In order to account for the relation between examined and infected subjects surveyed, Galen & Gambino (1975) introduced prevalence-related measures which provide (1) a value predicting the accuracy of positive results (PV_{pos}) which is the proportion of correctly diagnosed

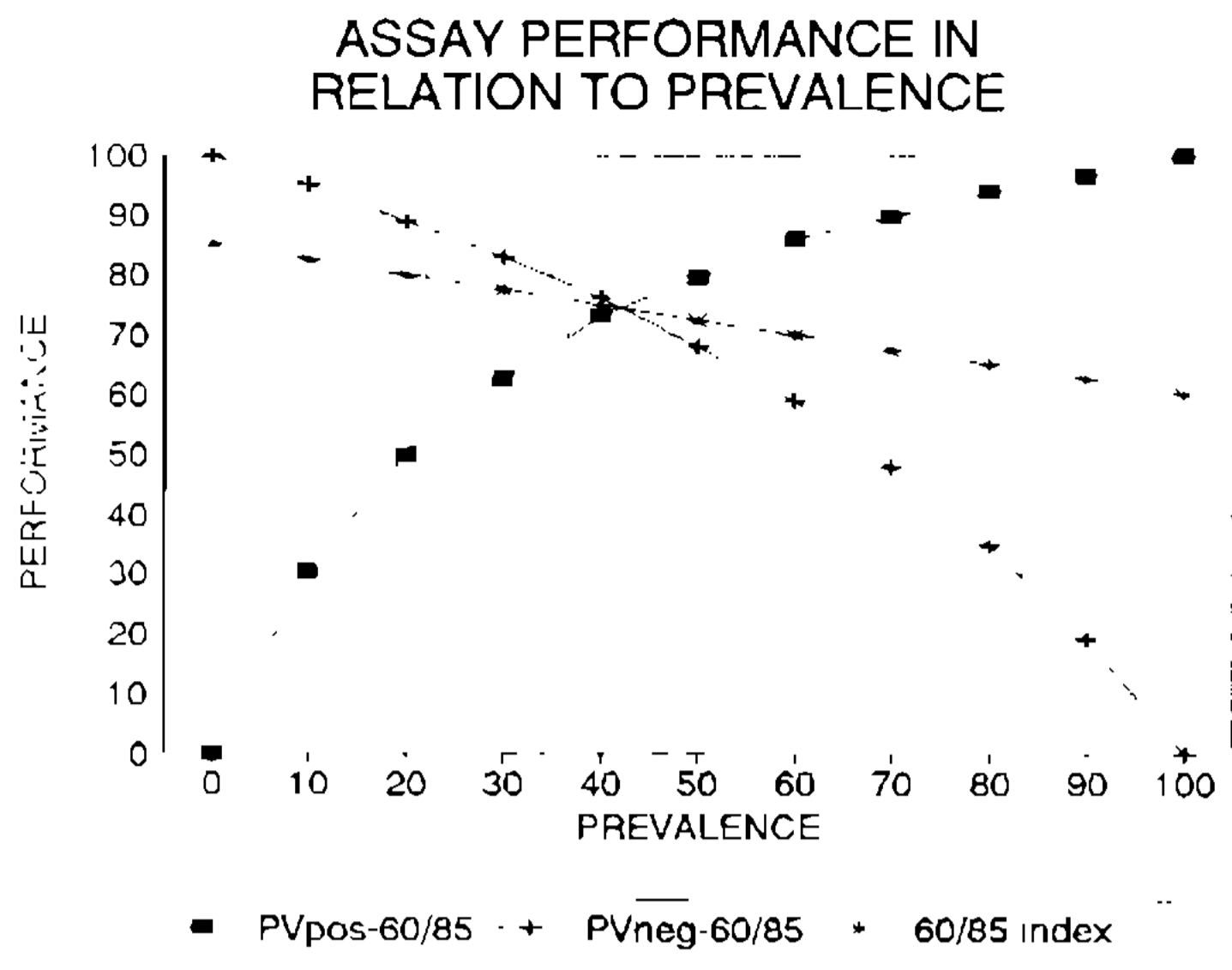


Fig. 3: the predictive positive (PV_{pos}), predictive negative value (PV_{neg}) and overall performance reliability of test results at different rates of prevalence of a hypothetical assay with the sensitivity to specificity ratio of 60/85.

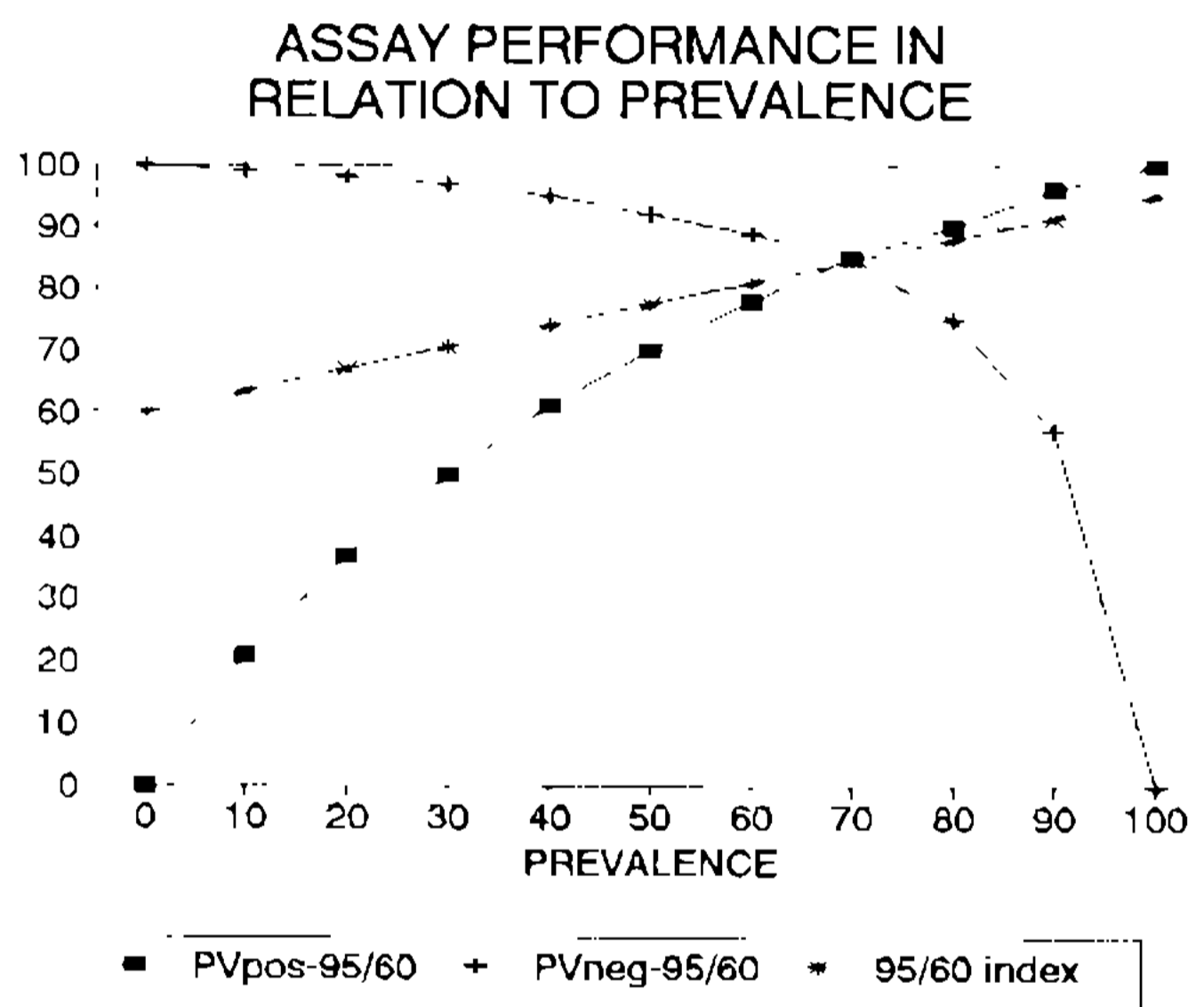


Fig. 4: the predictive positive value (PV_{pos}), predictive negative value (PV_{neg}) and overall performance reliability of test results at different rates of prevalence of a hypothetical assay with the sensitivity to specificity ratio of 95/60.

infected subjects out of all positive test results and (2) a value predicting that of negative test results (PV_{neg}) which is the proportion of truly negative subjects in relation to all negative test results. A consolidated value of all correct test results, positive and negative, in relation to the total number of tested subjects provides an overall test performance. The variation of this performance index with the prevalence is exemplified in Fig. 2 using three hypothetical assays with arbitrarily chosen values for sensitivity and specificity. However, rather than expressing the test reliability in the form of a performance index, the presentation of the negative and the positive prediction highlights the considerable influence of the prevalence on survey results at the extremes of the scale

(Figs 3, 4). For example, the PV_{pos} of an assay characterized by 60% sensitivity and 85% specificity (Fig. 3) applied in a population with a prevalence of 80%, indicates that the reported number of positive cases is 94% correct, whilst the reliability of the negative test results is only 35%. However, if the prevalence were instead no more than 10%, the PV_{pos} would be reduced to 30%, whilst the PV_{neg} would stand at 95%. If the test sensitivity were improved, for example by sacrificing its specificity by adjusting the cut-off, the main difference would be a decrease in falsely negative results including their virtual disappearance at 10% prevalence (Fig. 4).

Cross-reaction with antigens from other helminths whose endemicity coincides with that of schistosomes is a source of serious error (Corrêa-Oliveira et al., 1988). It is strictly a function of antigen specificity as opposed to assay specificity which is a composite value incorporating also other undesired reactions such as, for example, non-specific reactions due to the quality of the reagents used. Cross-reactivity varies independently of these parameters and may be tested by homologous and heterologous antigen inhibition. Immunoblot analysis may also be utilized for investigating antibody specificity which has been reviewed by Tsang et al. (1983). A miniaturized version of the immunoblot procedure does not require more than 50 μ l of serum per test (Nghiem, 1988).

DISCUSSION

The increased need for improved diagnostic procedures has revived an interest in serology at a time when rapid advances in molecular biology facilitate their development. Historically, antibody detection has worked well, for example as an adjunct to clinical diagnosis of the childhood viral infections, but in schistosomiasis and in other tropical parasitoses, the field of application is narrower since frequent reinfection makes antigen detection preferable. Furthermore, since stool and urine examinations are adequate in areas of medium to high prevalence there is no case to be made for serology until the intensity of infection has been considerably reduced. Due to the availability of safe and effective anthelmintic drugs, in contrast to the situation in diagnosis of the human immunodeficiency virus (HIV), a fair number of falsely positive results can be accommodated, whilst falsely negative results are

more disturbing. For this reason, although fundamental in areas of high prevalence, specificity is of secondary importance when surveying areas characterized by a low level of infection. The need to consider the predictive results at different rates of prevalence is amply illustrated in Fig. 4 where the overall performance index is actually decreasing towards the low-prevalence end where, from the point of view of control, the test would provide the most useful results. In Fig. 3, overall performance improves at low prevalence rates, whilst further analysis indicates that almost 10% falsely negative test results can be expected at 15% prevalence. These examples emphasize the importance of carefully determining assay parameters before their implementation in the field. This would be facilitated by the establishment of a reference specimen bank including regional collection centres contributing standardized clinical specimens from different endemic foci.

The presence of immune complexes may complicate determination of circulating parasite antigens by partially or even completely masking relevant epitopes. This risk would be increased in antibody excess which, due to the minute amounts of antigens released, is the most probable form of complex. The use of a battery of MAbs might improve test results by targeting different epitopes including determinants not expressed on all circulating antigens. In this way, sensitivity could be actually increased without sacrificing specificity.

The glomerular membrane limits the excretion of antigens to sizes normally not exceeding 60-70 kDa which, if the reported higher-range molecular weights of CCA reflects aggregations, explain why this antigen is more abundant in the urine than CAA. Excreted antigens have an advantage in relying on non-invasive means of specimen collection which poses less risk for accidental spread of infections such as viral hepatitis or HIV, a fact clearly signalling that research should be centered on such antigens.

CONCLUSIONS AND RESEARCH PRIORITIES

The study of diagnostic procedures in areas of different rates of prevalence has contributed to the emergence of a holistic view according to which serological and parasitological methods complement each other. The latter are useful for epidemiological surveys in areas of

presumed high prevalence, whilst antigen detection is necessary for the follow-up of chemotherapy. Testing for specific antibodies is satisfactory in the late maintenance phase and, if sufficiently short-lived antibodies are targeted, also during more active control activities. The integration of serologic methods into national control programmes would, however, first require successful large-scale trials of assays for antigen detection and standardization of serological techniques. In order to reach this goal a number of priority tasks including the following must be undertaken: (a) establishing a central specimen bank responsible for quality control, storage and distribution. The specific requirements would be to obtain and provide specimens together with essential clinical data; (b) cataloguing available tests for antibody detection and characterizing, immunologically and biochemically, useful diagnostic schistosome antigens; (c) cataloguing available specificities of MAbs used for antigen detection and investigating the advantage of combining sets of MAbs with different specificities; (d) investigating, in large-scale trials in humans, the usefulness of the currently most developed assays for antigen detection; (e) investigating the influence of immune complexes in assays for antigen detection; and (f) developing assays permitting quantitative determination of actual worm burdens for use in future human vaccine trials.

Immunodiagnostic surveys should be planned in collaboration between research laboratories, health service laboratories and epidemiologists, and assays should be defined in terms of sensitivity, specificity, predictive values and reproducibility using sera from clinically well-characterized subjects. Implementation of serological testing with standardized testing systems would permit comparisons between different geographical areas providing a sound base for decisions regarding national policies.

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