Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods

J. V. HAMILTON[1], M. KLINKERT[2] *and* M. J. DOENHOFF[1]*

[1] *School of Biological Sciences, University of Wales, Bangor, Gwynedd, UK, LL57 2UW.*
[2] *Institute of Cell Biology, Consiglio Nazionale delle Ricerche, 43 Viale Marx, 00137 Rome, Italy*

SUMMARY

Schistosomiasis remains a serious world-wide public health problem with a still unfulfilled need for routine cost-effective methods of diagnosis. Such methods are required not only for people in endemic areas, but increasingly for tourists who may have become infected during visits to such places. This article reviews the wide range of immunoassays and antigenic preparations that have been shown to have potential for diagnosis of schistosomiasis by the indirect method of antibody detection. Antigens in native form derived from cercariae, adult worms and eggs are considered, as well as schistosome antigens produced by recombinant DNA technology and the schistosome cross-reactive antigen, keyhole limpet haemocyanin (KLH). Respective advantages and disadvantages of antibody detection, circulating antigen detection and parasitological methods of diagnosis are analysed. It is suggested that due to the relative insensitivity of both parasitology and antigen detection, antibody detection methods could find increasing use in situations of low infection intensity.

Key words: schistosomiasis, diagnosis, immunodiagnosis, serology, *S. mansoni*, *S. haematobium*, *S. japonicum*, KLH.

INTRODUCTION

Methods allowing infections to be correctly diagnosed are a prerequisite for effective disease control and, with the incidence of schistosomiasis showing no decline, the need for good diagnostic tools is as great as ever. This is not just for use in endemic areas, but also for correct diagnosis of infection in travellers and tourists, increasing numbers of whom are returning home infected (Day *et al*. 1996; Visser, Polderman & Stuiver, 1995). This review is primarily concerned with immunodiagnosis of schistosome infections by the indirect method of detecting specific antibody reactivity. The potential advantages and disadvantages of antibody detection can, however, only be appreciated in the context of the relative efficacies of other available methods of diagnosis. The 'direct' methods of diagnosing schistosomiasis, either parasitologically or by detection of circulating and excreted antigens, are therefore first subjected to a brief survey. Other recent reviews on diagnosis of this parasitic infection include those by Tsang & Wilkins (1997), Rabello (1997), Feldmeier & Poggensee (1993) and Bergquist (1992), with earlier reviews by Kagan (1968) and Maddison (1987, 1991).

PARASITOLOGICAL METHODS OF DIAGNOSIS

Detection of ova is the traditional method of diagnosis of schistosomiasis, with the infecting species identified by egg morphology. It is the most direct and specific means by which the presence of a schistosome infection can be established. There are many variations in parasitological methods, but currently the most widely used method for detection of eggs of *Schistosoma mansoni* and *S. japonicum* in faeces is based on the Kato-Katz thick smear (Kato & Miura, 1954; Martin & Beaver, 1968; Katz, Chaves & Pellegrino, 1972). Filtration and other concentration methods (e.g. Ridley & Hawgood, 1956; Bell, 1963; Knight *et al*. 1976) have been devised to improve the sensitivity of faecal egg detection methods, and the more invasive rectal snip biopsies are used to some extent, again for heightened sensitivity. Filtration of urine using Millipore filters (Dazo & Biles, 1974), Nucleopore filters (Peters *et al*. 1976), Nytrel filters or filter papers (Braun-Munzinger, 1986) is the standard method for detecting excreted *S. haematobium* eggs.

It has been widely and increasingly recognized that parasitological methods of diagnosis lack accuracy and sensitivity (Ruiz-Tiben *et al*. 1979; Sleigh *et al*. 1982; Barreto, Smith & Sleigh, 1990; De Vlas & Gryseels, 1992; Gryseels & De Vlas, 1996; Ebrahim *et al*. 1997; Doenhoff, 1998*c*). Factors which contribute to this problem are large day-to-day variations in excreted egg counts in individual patients (Barreto *et al*. 1978; Engels, Sinzinkayo & Gryseels, 1996; Van Etten *et al*. 1997*a*) and uneven distribution of eggs in excreta (Engels *et al*. 1997; Ye *et al*. 1998). Other host- and parasite-related factors may also affect how accurately an excreted egg count reflects infection intensity. These include possible imbalances in adult worm sex ratios, the occurrence of immature infections, severe pathological lesions in host tissues (Cheever, 1968), the immune-dependence of schi-

* Corresponding author.

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

stosome egg excretion (Karanja *et al*. 1997; Doenhoff, 1998*a*), and the amount and consistency of excreted faecal matter, which may in turn be influenced by diet and body size. In urinary schistosomiasis worms may survive after an immunologically induced inhibition of fecundity (Agnew *et al*. 1992).

Errors due to an inherent lack of sensitivity in a diagnostic method are likely to be exaggerated when infection intensities are low, as in areas of low prevalence and in individuals in the earliest or latest stages of schistosome infection. The problem will thus be exacerbated particularly when the prevalence and intensity of schistosomiasis is being reduced through the introduction of effective control measures. In cases of imported (traveller's) schistosomiasis also, excreted eggs are likely to be low in number or absent due to exposure to cercarial contaminated water having been relatively limited.

Parasitological examination of faeces or urine has advantages of requiring only relatively unsophisticated equipment and basically trained personnel. It is likely therefore to be the lowest cost option particularly when technical assistance is plentiful. Problems of insensitivity can be compensated for by increasing the size and/or number of samples examined, or by resorting to tissue biopsy, although such alternatives will serve to increase cost and/or reduce convenience and patient compliance. Note should also be taken of calculations indicating that if, for example, a single stool examination shows a population to have a prevalence of $\sim 40\%$, four further examinations will still result in the true prevalence being underestimated by 20% (De Vlas & Gryseels, 1992). For epidemiological purposes mathematical modelling may allow infection intensities to be estimated more accurately from parasitological data (De Vlas *et al*. 1993), but there is no advantage here for the clinician who requires accurate diagnostic results on which to base individual treatment decisions.

Some of these difficulties may be overcome by the adoption of an immunodiagnostic technique.

DIAGNOSIS OF SCHISTOSOMIASIS BY DETECTION OF CIRCULATING SCHISTOSOME ANTIGENS

Okabe & Tanaka (1961) were perhaps the first to observe that antigens secreted or excreted by the parasite may have potential for diagnosis of infection. Others, including Berggren & Weller (1967), Nash, Prescott & Neva (1974) and Deelder *et al*. (1976) subsequently produced confirmatory evidence for the presence of a schistosome-derived antigen in the circulation of infected experimental animals. The antigen was a proteoglycan found in the adult parasite's gut caecum and the epithelial cells lining it – hence one of the early names for circulating antigen(s) was gut-associated schistosome proteoglycan or GASP – and it migrated anodally in electrophoresis, from which property derives the name by which it is now generally known, i.e, circulating anodic antigen (CAA). A second schistosome-derived, cathodally migrating antigen (circulating cathodic antigen, CCA), was also found in serum and urine of experimentally-infected animals (Deelder *et al*. 1976). This antigen was antigenetically distinct from CAA, and was subsequently equated with antigen 'M' that had been found in the serum, urine and milk of *S. mansoni*-infected patients (Carlier *et al*. 1975; Santoro *et al*. 1977). CAA and CCA are both proteoglycans, and their respective physicochemical properties and structure have been partially elucidated (Nash *et al*. 1974; Deelder *et al*. 1980; Bergwerff *et al*. 1994; van Dam *et al*. 1994, 1996). Panels of monoclonal antibodies raised against these antigens have facilitated elucidation of their respective immunological properties and their localisation in or on the parasite (Deelder *et al*. 1996; Bogers *et al*. 1995). An important characteristic in the present context is the presence of repeated (glycanic) epitopes on both of these molecules. It is perhaps also worth noting that the antigenicity of these circulating antigens is not species specific, with antibodies raised against the respective *S. mansoni* antigens showing reactivity against *S. japonicum*, *S. haematobium* and other schistosome species (de Jonge *et al*. 1989; van't Wout *et al*. 1995; Clercq *et al*. 1995; Agnew *et al*. 1995; van Etten *et al*. 1997*a*). The role of these proteoglycans in the biology of the host-parasite relationship has, however, yet to be fully elucidated.

There is evidence for the presence of other parasite-derived antigens in the circulation of infected hosts, including schistosome worm antigens of 31/32 kDa (Li *et al*. 1994, 1996) and 70 kDa (Chi & Carter, 1990), 28 kDa glutathione S-transferase of *S. japonicum* (Davern *et al*. 1990) and antigens bearing a repeating carbohydrate epitope expressed in all *S. mansoni* developmental stages (Hassan, Badawi & Strand, 1992).

The existence of detectable quantities of parasite-derived antigens in the circulation and excreta of infected hosts has prompted considerable research on their potential for immunodiagnosis of schistosomiasis. A variety of assay methods have been used to detect circulating antigens, including indirect haemagglutination (Deelder *et al*. 1989*b*), time-resolved immunoflurometric assay (de Jonge, Boerman & Deelder, 1989), magnetic bead immunoassay (Gunderson *et al*. 1992) and reagent strips (van Etten *et al*. 1997*b*). Antigen detection assay methods generally involve capture of the antigen in an antibody 'sandwich', the antibodies being monoclonal or polyclonal with specificity for repeated epitopes on the antigens. A currently on-going project has the objective of exploiting the 'compact

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

disc' (CD) as a platform on which to perform immunoassays such as this (John Kusel, personal communication).

Although unable to distinguish between infections of different schistosome species, diagnosis by detection of CAA and CCA is relatively specific; i.e. little false positive reactivity is found in non-endemic or endemic areas. In *S. mansoni*-infected subjects the sensitivity of CAA detection by antigen capture ELISA was estimated to compare well with a single faecal egg count (de Jonge *et al*. 1988), with the lower level of antigen detectability in serum corresponding to an egg excretion rate of 10 eggs/gram (epg) faeces (Deelder *et al*. 1989*a*). There is generally a positive correlation between the circulating antigen levels and schistosome egg excretion rates (Deelder *et al*. 1989*a*; de Jonge *et al*. 1991; de Clercq *et al*. 1995; van Lieshout *et al*. 1995*b*). The sensitivity of antigen detection methodology tends, however, to be poorer in situations where prevalence and/or intensity of infection are lower (de Jonge *et al*. 1988; van Lieshout *et al*. 1995*a*). Results from parallel testing for both CCA and CAA detection in both serum and urine results in improved diagnostic performance (van Lieshout *et al*. 1992). Higher CCA titres may occur in patients with hepatosplenic schistosomiasis than in those with intestinal disease, while there was no such difference with CAA (de Jonge *et al*. 1991).

When compared with other diagnostic methods and antibody detection in particular, detection of schistosome-derived antigens has several potential advantages. Both CAA and CCA can be detected in urine (Disch *et al*. 1997; van Etten *et al*. 1996), the collection of which is less invasive than blood/serum collection, with testing for CCA in urine being perhaps somewhat more sensitive than for CAA (van Lieshout *et al*. 1995*a*). The presence of parasite-derived antigens is considered to be a more accurate indicator of active infection, thus enabling the effects of treatment to be assessed more effectively (Hassan, Badawi & Strand, 1992; van Lieshout *et al*. 1994; de Clercq *et al*. 1997).

The putative advantages of antigen detection methods over antibody detection are to some extent comprised by an extra step in the immunoassay procedure, the necessity for pretreatment of serum and urine samples prior to use (de Jonge, Fillie & Deelder, 1987; Krijger, van Lieshout & Deelder, 1994), and a requirement for special filter papers when these are used for blood collection and storage (Jamaly *et al*. 1997).

DIAGNOSIS OF SCHISTOSOMIASIS BY ANTIBODY DETECTION

The principal requirements of a diagnostic test are that it should be both 'sensitive' and 'specific, each of which terms is mathematically well-defined (Feinstein, 1975). Fig. 1 shows how the result of an indirect diagnostic test is related to the occurrence of disease and how specificity and sensitivity are calculated. From the results of an indirect test (such as antibody detection) 'predictive values', both positive and negative, can be calculated, these being of use in determining the probability of disease being present or absent. Predictive values are determined by the sensitivity and specificity of the test and take into account the prevalence of the disease in the population being tested. (See Fletcher, Fletcher & Wagner, 1996, or other comparable sources for more detailed explanations of these terms.)

There is generally a trade-off between sensitivity and specificity, and the relative performance of a new test is therefore often calculated as an index in which both of these parameters are taken into account. Furthermore, as stated by Fletcher *et al*. (1996), 'assessment of the test's accuracy rests on its relationship to some way of knowing whether the disease is truly present or not – a sounder indication of the truth (being) often referred to as the 'gold standard'. As it turns out, the gold standard is often elusive.'

It will be seen that this problem acutely affects antibody detection tests for schistosomiasis because the 'gold standard' for comparison almost invariably has had to be parasitology. As suggested above, parasitology is itself defective due to its insensitivity, and a consequence of this may be an apparent poor specificity (i.e. high proportion of false positive results) given by antibody detection tests.

Assay system

Patent schistosome infections are highly immunogenic and there is generally no difficulty in demonstrating the presence of specific anti-schistosome antibody or cell-mediated immune responsiveness in infected subjects. In the search for a sensitive, specific and cost-effective immunodiagnostic test many different systems have been examined, including hypersensitivity reactions to intradermally-introduced antigen, complement-fixation tests (CFT), indirect fluorescent antibody tests (IFAT), indirect haemagglutination (IHA), radioimmunoassay, various flocculation tests, the circumoval precipitin test (COPT) and the cercarienhullen reaction (CH): see reviews by Kagan (1968) and Maddison (1987).

Currently, the most widely used diagnostic system is the enzyme-linked immunosorbent assay (ELISA), first described by Engvall & Perlman (1971). 'Western blotting' and 'dot-blotting' are variations of this type of methodology in which a secondary enzyme/anti-immunoglobulin conjugate is used to detect the presence of primary antibody reactivity against the parasite antigen(s). Since the introduction of the microtitre plate-based ELISAs (Voller, Bartlett & Bidwell, 1976; Voller *et al*. 1974)

		DISEASE	
		Present	Absent
TEST	Positive	True positive a	False positive b
	Negative	False negative c	True negative d

$$\text{Sensitivity} = \frac{a}{a+c} \qquad \text{Specificity} = \frac{d}{b+d}$$

Sensitivity = the number of people who give a positive test result as a proportion of the total number who have the disease.

Specificity = the number of people who give a negative test result as a proportion of the total number without the disease.

Fig. 1. Diagram to illustrate the relationship between the result of a diagnostic test and the presence of disease, including definitions of the diagnostic terms 'sensitivity' and 'specificity', and equations by which they are calculated.

the test has been deployed very widely for detection and quantification of specific antibodies. Venkatesan & Wakelin (1993) have summarized the practicalities of ELISA and how its application to diagnosis of parasitic infections may be optimized.

In comparative studies on anti-schistosome antibodies, ELISA has been demonstrated to be a more satisfactory test system than IFAT (Huldt, 1975; Hassan *et al*. 1979; Deelder & Kornelis, 1980; 1981; Evengard, 1990), IHA (Hassan *et al*. 1979; Deelder & Kornelis, 1981), CFT (McLaren *et al*. 1978) and COPT (Tanabe *et al*. 1990). ELISA also performed more satisfactory than IFA (Deelder & Kornelis, 1980), a defined antigen substrate sphere test (Deelder *et al*. 1977) and immunoelectrophoresis (Deelder & Kornelis, 1981).

In 2 interlaboratory trials completed under the auspices of the World Health Organization (Mott & Dixon, 1982; Mott *et al*. 1987), the performance of ELISA and a variety of other methods were directly compared on the same panel of test sera. The results did not indicate that any particular method for detecting antibody activity was superior.

Variations of ELISA have been developed and tested. Thus, a kinetic-dependent modification (k-ELISA; Tsang *et al*. 1982) was evaluated in the WHO multi-centre trial on immunoassays for *S. japonicum* (Mott *et al*. 1987), but in that instance k-ELISA performed less satisfactorily than conventional ELISA. Hancock & Tsang (1986) developed the Falcon assay screening test system (FAST-ELISA), using microsomal adult worm antigens (MAMA). More recently, Tsang & Wilkins (1997), working at the Centers for Disease Control, Atlanta, USA, have proposed use of FAST-ELISA as an initial diagnostic screening test, with Western blotting for species-specific confirmation. Both assays involve detection of antibodies reactive against adult worm antigens and the cost estimates and commercial availability of the test systems are also given (Tsang & Wilkins, 1997).

Moser, Doumbo & Klinkert (1990) developed the transferable solid phase (TSP)-ELISA based on the principle of the FAST-ELISA. This system allowed the assessment of *in vitro*-expressed amino-acid fragments of 3 cloned adult worm antigens, heat shock protein 70 (Moser *et al*. 1990) and Sm31 and Sm32 (Klinkert *et al*. 1991) for the diagnosis of schistosomiasis. In neither the study by Moser *et al*. (1990) nor that by Klinkert *et al*. (1991) was good sensitivity demonstrated, but it remains unclear whether this was due to the immunoassay or the antigen.

The dot-ELISA or dot-blot, involving performance of the ELISA test on antigens that have been blotted onto nitrocellulose paper, has been proposed as more appropriate for diagnosis of schistosomiasis in endemic areas (Boctor *et al*. 1987). When compared with IHA and COPT, dot-ELISA gave good sensitivity and adequate specificity (El Missiry *et al*. 1990). A dot-dye-immunoassay (dot-DIA) has also been used for the diagnosis of schistosomiasis; it was considered sensitive, rapid and practical (Rabello *et al*. 1992).

Ismail *et al*. (1989) compared the performance of the diffusion-in-gel ELISA (DIG-ELISA) against the conventional ELISA. This method allows blood spots on filter paper to be used directly without elution: after a period of diffusion the diameter of the rings of reactivity are indicative of the concentration of antibody. The DIG-ELISA performed as well as the conventional ELISA with *S. mansoni* and *S. haematobium* infections using both *S. mansoni* and *S. haematobium* egg and worm antigens.

There is thus a wide range of assays available for displaying antibody reactivity. Given satisfactory sensitivity and specificity, an important factor affecting choice of assay will be its cost, though the

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

influence of cost may depend on the objective: for example, it may be less of a concern for practitioners in traveller's medicine than for epidemiologists.

Choice of antigen

Antigens from virtually all stages of the schistosome life cycle have, at one time or another, been tested for immunodiagnostic potential. Some immunoassays use more or less intact morphological forms of the parasite: for example, immunoprecipitation on cercariae in the CH reaction, IFAT on larval or adult worm sections and the COPT on schistosome eggs. While many different methods of assaying antibody reactivity may offer similarly high degrees of sensitivity and specificity, the same claim can not be made for different schistosome antigens. There follows a consideration of the relative merits of schistosome larval, adult worm and egg antigens, including some that have been purified from the different stages, or produced by recombinant DNA technologies.

Larval antigens

Schistosome larval antigens have seldom been recommended for serodiagnosis as they have not given the levels of sensitivity and specificity obtained from adult worm or egg antigen preparations. This may be due to the relatively short periods that hosts are actually exposed to larval antigens during the course of an infection and also to the potentially poor immunogenicity of migrating larvae. The possibility that larval antigens may be useful for discriminating between acute and chronic infections has, however, been noted. Thus, Gazzinelli *et al*. (1985) used ELISA to compare the antibody responses of 15 Brazilian patients suffering from acute *S. mansoni* infections with responses of chronically infected patients matched for age and infection intensity. Patients with acute infections were more reactive to cercarial antigens than worm antigens. Reactions to egg antigens in acutely infected patients were intermediate in magnitude compared with cercarial and worm antigens. In another comparison of acutely and chronically infected *S. mansoni* patients the level of response to cercarial antigen was higher than that to crude adult worm antigen in the acute patients (Lunde & Ottesen, 1980), and a cercarial to adult worm ratio (C:A) clearly differentiated acute from chronic schistosomiasis. Deelder & Kornelis (1981) were, however, unable to demonstrate such a clear-cut difference between acute and chronic patients using the C:A ratio.

The pre-acetabular gland secretions of *S. mansoni* cercariae contain a 30 kD proteinase that shows homology with rat pancreatic elastase (Newport *et al*. 1988), and infected humans and mice produce antibodies that react with this antigen (Pino-Heiss *et al*. 1986). Toy *et al*. (1987) showed that this proteinase (cercarial elastase) could be used to distinguish *S. mansoni*-infected patients from controls. Both IgG and IgM were detected, but the greatest discrimination was found measuring IgG. Sera from patients with *S. haematobium* and *S. japonicum* were, however, also reactive against the *S. mansoni* elastase, suggesting that this antigen would not be useful for diagnostic discrimination between schistosome species. Recent work has indicated that the presence of antibodies reactive against the purified elastase may serve as a marker of exposure to infection (Ramzy *et al*. 1997). However, possible antigenic cross-reactivity between the proteases of different species may limit even this use in areas endemic for heterologous non-human schistosome species which produce larvae that can penetrate human skin.

Worm antigens

Of the different life-cycle stages of schistosomes found in the definitive host, adult worms are advantageous in being the most abundant and easily obtained source of antigenic material. Furthermore, crude schistosome worm antigen preparations are more reactive with infected patients' sera, and yield greater levels of diagnostic sensitivity and specificity than larval antigens (McLaren *et al*. 1978; Lunde, Ottesen & Cheever, 1979).

While crude worm antigen preparations have proved efficacious in ELISA (Maddison *et al*. 1985), it has often and reasonably been supposed that purified antigen preparations will give higher levels of diagnostic sensitivity and specificity than crude extracts. Purified worm antigens may also provide diagnostically useful information that is not available after use of crude antigen mixtures alone, for example, relating to age of infection, distinct pathologies or protective immunity. The degree of purification of worm antigens varied between different studies. Thus, tegumental antigens extracted with 3M KCl were used in ELISA to detect total antibodies to schistosomiasis in a cohort of 559 Ethiopian patients and relative to the result from Kato thick smear examinations the sensitivity and specificity of the serological tests were 99·0% and 87·5% respectively (Jemaneh, 1993).

A *S. mansoni* worm microsomal antigen preparation (MAMA; Tsang *et al*. 1983*a*, *b*) is being used in ELISA and 'Western' immunoblotting for routine diagnosis in the Centers for Disease Control, Atlanta, USA (Tsang & Wilkins, 1997). Other worm preparations that have been used for diagnosis include gut-associated polysaccharide (GASP) antigens (Deelder & Kornelis, 1981; Nash, Lunde & Cheever, 1981), trichloroacetic acid fractions of adult worms (Deelder *et al*. 1980; Nash *et al*. 1981), excretory-secretory adult worm antigens (Yacoub & Lillywhite, 1985), phenol sulphuric test active peak

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

(PSAP) (Nash *et al*. 1983), heat shock protein 70 (Moser, Doumbo & Klinkert 1990), a 38 kDa adult worm antigen (Newport *et al*. 1988), worm antigens Sm31 and Sm32 (Ruppel *et al*. 1990; Klinkert *et al*. 1991), and worm-derived cysteine proteinases (Chappell *et al*. 1990).

An interesting variation on enzyme-immunoassay has been devised to exploit both the antigenicity and enzymatic activity of *S. mansoni* adult worm alkaline phosphatase (Pujol, De Noya & Cesari, 1989; Pujol & Cesari, 1990). The technique involves quantifying the enzymatic activity of parasite alkaline phosphatase (AP) that is captured onto the surface of micro-titre wells by initial layers of Protein A and patients' IgG anti-AP antibody. The test was 100% specific in an area without *S. mansoni* transmission and 89% sensitive in an endemic area where 69% of the subjects excreted less than 100 epg faeces, but it was less sensitive in children under 5 years of age who had a positive COPT test (De Noya *et al*. 1997). Alkaline phosphatase derived from adult *S. mansoni* worms was shown to be schistosome species-specific, but *S. mansoni* infections from different geographical areas could not be distinguished by this method (Cesari *et al*. 1998).

Analysis of antibody reactivity by immunoglobulin class and subclass may give more information about the stage of the infection. Thus, patients with acute infections had higher IgA and IgM anti-worm ELISA titres than those who were chronically-infected (Valli *et al*. 1997). The appearance of IgM, IgG (subclasses IgG_1, IgG_3) and IgA_1 against adult worm preparations have been investigated for the early diagnosis of schistosomiasis: IgG_1 and IgG_3 antibodies recognized 32–35 kDa antigens in an adult worm preparation and it has been suggested that antibodies against these antigens could be a marker of early infection in previously non-exposed visitors to endemic areas (Evengard *et al*. 1990). Relative reactivities against particular antigenic peptide sequences of individual molecules may also allow differentiation between acute and chronic infection, as has been found using recombinant *S. mansoni* glutathione S-transferase (GST; Evengard *et al*. 1994).

Differentiation between class and subclass antibodies may provide information regarding a subject's level of resistance to schistosome re-infection. Thus, Satti *et al*. (1996*a*) found an association between anti-worm IgE and IgG_1 antibodies to whole worm antigen and resistance in canal cleaners in Sudan.

Some more sophisticated assays have been tested for their usefulness in immunodiagnosis; for example, a glass microfibre histamine release method has been tested on *S. mansoni*-infected individuals from schistosome non-endemic areas (Satti *et al*. 1996*b*). The method uses patients's sera to passively sensitize basophils and induce a positive histamine release in response to whole worm or egg antigens. Worm antigens induced a significantly higher histamine release in adult patients than egg antigens. In addition, basophil cell sensitivity to worm homogenate was inversely related to the intensity of infection (Satti *et al*. 1996*b*). The increased cost incurred in performing these assays is, however, likely to limit their usefulness for routine diagnosis.

Egg antigens

Observations on experimentally infected hosts have not unexpectedly shown that reactivity against egg antigens remain at a low level before infections become patent (Dunne *et al*. 1984). However, antibodies specific for antigens in earlier stages of the infection are also minimal before infection patency (Ambroise-Thomas & Andrews, 1976). Infecting schistosome larvae and pre-patent adult worm stages therefore seem to lack immunogenicity and this may help to explain why so far there is no satisfactory immunological method for diagnosing human schistosome infections before they become patent. The 'blocking antibody hypothesis' of schistosome immunity provides one explanation for the increase in anti-larval and anti-worm antibody reactivity when infections become patent; i.e. following patency the first antibody responses are directed against carbohydrate epitopes of immunogenic egg glycoproteins. Certain immunoglobulin isotypes (e.g. IgG_2) may be more involved than others in early responses (Langley *et al*. 1994) and these antibodies may cross-react with glycanic epitopes that are also present, though in less immunogenic form, on the infecting larval and adult worm stages (Doenhoff, 1998*b*). A similar explanation may account for the reactivity of mouse and human infection sera against *S. mansoni* worm neutral glycolipids (Dennis *et al*. 1996).

Results from both individual laboratories and multicentre trials suggest that crude extracts of schistosome eggs yield higher levels of sensitivity and specificity than crude worm extracts (McLaren *et al*. 1978; Lunde, Ottesen & Cheever, 1979; Mott & Dixon, 1982; Mott *et al*. 1987; Khalil *et al*. 1989). In one detailed study Tanabe *et al*. (1990) examined *S. mansoni* patients from Brazil using a number of methods and found ELISA using *S. mansoni* SEA to be the most sensitive assay (98%), followed by ELISA with *S. mansoni* worm antigens (82·4%). COPT with fixed *S. mansoni* eggs and immunodiffusion had lower sensitivities of 72·8% and 58·8% respectively. The specificity with ELISA employing either egg or worm antigens was 97% using 37 sera of which 71% were positive for at least one species of parasite other than schistosomiasis.

ELISA with unfractionated extracts of *S. mansoni* eggs has been recommended for clinical diagnosis of schistosomiasis in travellers (Tosswill & Ridley, 1986; Evengard, 1990) and is in routine use in the

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

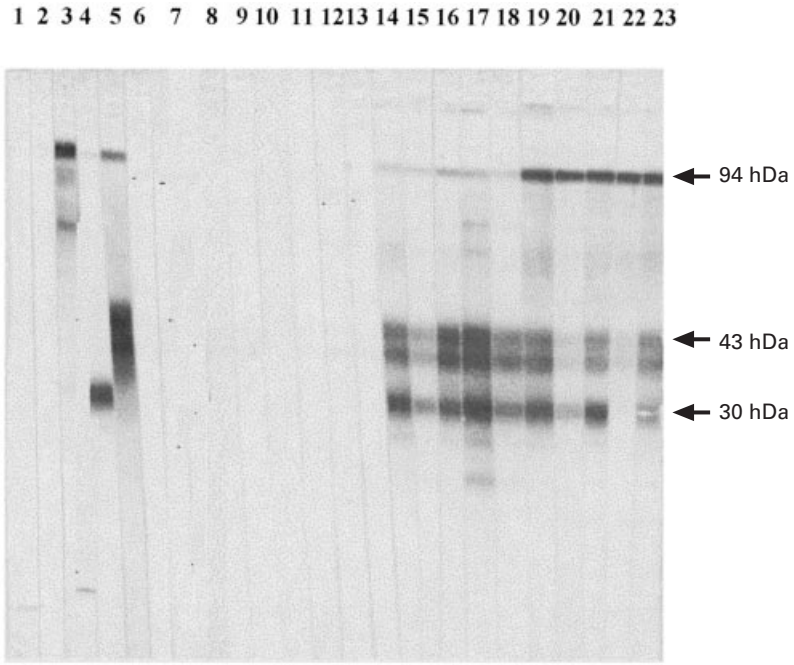

Fig. 2. Western immunoblot of *Schistosoma mansoni* egg antigens probed with monospecific rabbit antisera and human infection sera. Methods and rabbit antisera prepared as in Dunne *et al*. (1986). Lanes 1 and 2, human tropical non-infection control sera; 3, rabbit anti-κ5; 4, rabbit anti-ω1; 5, rabbit anti-α1; 6–13, human non-schistosome parasite infection sera; 6, cutaneous leishmaniasis; 7, *Trypanosoma cruzi*; 8, *Giardia lamblia*; 9, amoebiasis; 10, *Plasmodium falciparum*; 11, cysticercosis; 12, filariasis; 13; fascioliasis; 14–23, *S. mansoni* egg-positive, ELISA serology-positive patients.

parasitology departments of hospitals in the UK and elsewhere (P. L. Chiodini, H. Smith and R. O'Brien personal communications). Egg antigens have also been usefully exploited in more specialized clinical circumstances such as diagnosis of myeloradiculopathy (Ferrari *et al*. 1995) and in other clinical and epidemiological contexts (Eltiro, Ye-Ebiyo & Taylor, 1992; Xue *et al*. 1993; Doenhoff *et al*. 1993; Kamal, Shaheen & El Said, 1994; Gryseels *et al*. 1994; Van Dam *et al*. 1996; Li *et al*. 1997; Ghandour *et al*. 1997).

The use of partially purified egg antigens in ELISA may provide even greater immunodiagnostic specificity and sensitivity than crude egg antigen preparations. For example, CEF6 is a fraction containing 2 cationic antigens ω-1 and α-1 which is easily purified from *S. mansoni* egg homogenate (SEA) by cation exchange chromatography (Dunne *et al*. 1981, 1984; Dunne, Jones & Doenhoff, 1991). It was more specifically reactive for *S. mansoni* infection than SEA with respect to sera from patients with avian cercarial dermatitis and *S. haematobium* and *S. japonicum* infections (McLaren *et al*. 1981). Fig. 2 shows that in Western immunoblots antibodies from human subjects infected with *S. mansoni* react relatively intensely against the 2 antigens in CEF6, as well as against a third *S. mansoni* egg antigen identified as κ-5 (Dunne *et al*. 1986). It has been suggested that α-1 and ω-1 are immunoprecipitated components of the circumoval precipitin test (Dunne, Hillyer & Vasquez, 1988) and when tested in a multicentre trial against sera from a parasitologically well-defined panel of patients CEF6 gave sensitivity and specificity values of 91% and 90% respectively (Mott & Dixon, 1982).

The relationship between CEF6 and MSA_1, a *S. mansoni* egg glycoprotein found to have serodiagnostic potential in radioimmunoassay (Hamburger, Pelley & Warren, 1976; Pelley, Warren & Jordan, 1977), is unclear, though like CEF6, MSA_1 was shown to be involved in the COPT reaction (Hillyer & Pelley, 1980).

Recombinant antigens for immunodiagnosis of schistosome infection

Several cloned schistosome proteins have been shown to be antigenically reactive with human infection sera in immunoassays. So far, only a few have been tested for their diagnostic potential in ELISA of human sera, but these include P28 glutathione S-transferase (Auriault *et al*. 1990), a serine proteinase inhibitor-like molecule (Li *et al*. 1995), *S. mansoni* heat shock protein 70 (Moser *et al*. 1990), tropomyosin (Xu *et al*. 1991), and *S. mansoni* cathepsins B and L (Grogan *et al*. 1997*b*). Protein Sm31 (cathepsin B), together with Sm32 (an asparaginyl proteinase) first attracted attention because of their ability to induce early and strong antibody responses in experimental animals and humans (Ruppel, Diesfeld & Rother, 1985). These authors demonstrated satisfactory specificity and sensitivity

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

when used for the detection of infections with *S. mansoni* (Ruppel *et al*. 1985), *S. japonicum* (Ruppel *et al*. 1987) and *S. haematobium* (Idris & Ruppel, 1988). Recombinant fusion proteins Sm31 and Sm32 were produced (Klinkert, Ruppel & Beck, 1987) and shown to be promising diagnostic antigens when tested in Mali (Klinkert *et al*. 1991) and Egypt (El Sayed *et al*. 1998). A recombinant form of a 22·3 kDa antigen has been found usefully reactive with antibodies induced by *S. mansoni* and *S. haematobium* infections (Hancock *et al*. 1997). Additionally, an immunoreactive egg-specific protein, SmE16, has also been cloned and expressed in *Escherichia coli* (Moser, Doenhoff & Klinkert, 1992).

Recently, protocols were developed for the production of purified Sm31, Sm32 and SmE16 in unlimited amounts and in their native forms (Ewald Beck, personal communication). Soluble Sm31 was expressed in *Saccharomyces cerevisiae* cells (Lipps, Fullkrug & Beck, 1996), while SmE16 and Sm32 were synthesized in bacterial cells. Sm32 was produced first as an insoluble protein in urea and thereafter refolded to its native form *in vitro*. Subsequently, the immunoreactivities of all three *S. mansoni* recombinant antigens were examined using a simple immunoblot technique for the detection of antibodies in peripheral blood. Sera from African patients infected with *S. mansoni* and *S. haematobium*, as well as a small number of *S. japonicum* patients' sera were tested. Results obtained so far are very encouraging in that recombinant Sm32 was found to react equally with sera from *S. mansoni*, *S. haematobium* and *S. japonicum* patients with $> 90\%$ sensitivity. SmE16 also reacted with $> 90\%$ sensitivity with *S. mansoni* and *S. haematobium* sera, but with lower sensitivity ($< 50\%$) using *S. japonicum* sera. By combining recombinant antigens Sm32 and SmE16, *S. mansoni* and *S. haematobium* infections can be detected with $> 98\%$ sensitivity. Results with Sm31 were more disappointing since it reacted with all 3 schistosome species with a 50% sensitivity. Work is in progress to improve Sm31 reactivity and to adapt the immunoassay to field conditions. Trials in the southern part of China using Sj31 and Sj32 isolated from adult *S. japonicum* parasites led to $>$ 98% sensitivity (Wang, Zeng & Yi, 1995). Recombinant proteins Sj31 and Sj32 have been produced (Merkelbach *et al*. 1994) and sufficient quantities should become available for testing diagnostic potential.

Two further points are worth noting. First, recombinant proteins produced in microbial expression systems may not have the same configuration as the native molecule, and the general failure of such systems to produce glycosylated proteins may be particularly important. The use of alternative eukaryotic gene expression systems such as yeast or insect cells may overcome this problem. Secondly, difficulties in separating the expressed product of the schistosome gene from other constituents of the expression system, particularly microbial ones, may result in the recombinant antigen displaying poor specificity in immunoassays. As indicated above, schistosome glycoproteins, especially those present in the egg stage of infection, are particularly immunogenic and the diagnostic efficacy of many of the antibody detection assays that have been described may in part be due to antibodies which are specifically reactive with glycanic epitopes. It is perhaps because of this that the heavily glycosylated keyhole limpet haemocyanin has been found to have some immunodiagnostic value.

A non-schistosome antigen : keyhole limpet haemocyanin

Antibody activity against haemocyanin from the keyhole limpet, *Megathura crenulata* (KLH), has shown some potential for the diagnosis of *S. mansoni* infections in Brazil (Alves-Brito *et al*. 1992), *S. mansoni* and *S. haematobium* infection in Egypt (Markl *et al*. 1991), *S. haematobium* on Pemba Island, Tanzania (Xue *et al*. 1993) (Xue *et al*. 1993) and *S. japonicum* infections in China (Yuesheng *et al*. 1994; Li *et al*. 1997). A use for KLH has also been indicated in differentiation between acute and chronic schistosomiasis mansoni (Mansour *et al*. 1989; Rabello *et al*. 1993), acute and chronic *S. japonicum* infections (Zheng *et al*. 1992; Yuesheng *et al*. 1994; Li *et al*. 1997) and for the diagnosis of acute *S. mansoni* (Alves-Brito *et al*. 1992). Antibody responses to SEA and KLH have been used to assess chemotherapeutic cure for up to 2 years post-chemotherapy. Anti-KLH IgM activity became negative 2 months after treatment, and anti-KLH IgG declined between 12–24 months after treatment, while IgM and IgG antibody reactivity against *S. mansoni* worm and egg antigens remained positive during the follow up period (Rabello *et al*. 1997).

Whilst the above studies have indicated that KLH may be a useful diagnostic antigen in countries where schistosomiasis is endemic (Alves-Brito *et al*. 1992; Markl *et al*. 1991; Yuesheng *et al*. 1994; Mansour *et al*. 1989) another study suggested that KLH may not be as useful in a non-endemic clinical setting. Thus, Verweij *et al*. (1995) reported that KLH, when used in ELISA, could not discriminate between acute (3 month) or chronic (15 month) *S. mansoni* or *S. haematobium* infections in Dutch travellers returning from Mali, and that there was no significant difference in the anti-KLH response before and one year after chemotherapy with praziquantel. Similar observations were described by Markl *et al*. (1991) who reported that the anti-KLH response was similar in acute and chronic patients. Furthermore, as the references cited above indicate species-specific diagnosis of schistosomiasis is unlikely to be obtainable with KLH.

KLH and schistosomes have carbohydrate epitopes in common (Grych *et al*. 1987). We have suggested elsewhere that the apparent usefulness of KLH as a diagnostic agent for *S. mansoni* infection may be primarily due to immunological cross-reactivity between carbohydrate epitopes on KLH and *S. mansoni* egg antigens α-1, ω-1 and κ-5 which have proven immunodiagnostic potential (Hamilton *et al*. 1999).

One factor which may in the future limit the usefulness of KLH as a diagnostic aid for schistosomiasis is the use of it as a carrier molecule for immunisation with haptens, for example, in anti-cancer therapy (Livingston, 1995).

SOME ADVANTAGES AND DISADVANTAGES OF DIAGNOSIS BY ANTIBODY DETECTION

One of the criticisms most frequently levelled against diagnosis by antibody detection is that because the evidence is only indirect the result cannot accurately reflect the presence of active infection. Results from antibody detection methods are thus considered potentially flawed when compared with the more direct evidence of eggs in excreta or schistosome-derived antigens in blood or urine. The perceived defectiveness of antibody detection is deemed to be a particular problem with regard to monitoring the curative effects of chemotherapy.

Excreted egg counts and circulating antigen concentrations do indeed decline relatively rapidly after chemotherapy (Deelder *et al*. 1994). However, this parameter in antibody detection methods may be improved with the use of purified antigens or by discriminating between immunoglobulin isotypes. Thus, following chemotherapy of *S. mansoni* infections anti-CEF6 antibody titres decayed more rapidly than anti-SEA antibodies (Mott & Dixon, 1982; Dunne, Hillyer & Vasquez, 1988; Doenhoff, Dunne & Lillywhite, 1989). In other studies the decrease in egg excretion rates and in circulating antigen concentrations was associated with a substantial drop in IgG_4 anti-SEA antibodies (Grogan *et al*. 1996) and IgA anti-SEA antibody titres decayed more rapidly than IgM and IgG antibodies (Rabello *et al*. 1997).

A common criticism of antibody detection is that titres do not correlate well with infection intensity. As with post-chemotherapy monitoring, however, use of a purified antigen fraction in the form of CEF6 (Mott & Dixon, 1982), or assay of anti-SEA antibodies by IgG_4 isotype (Grogan *et al*. 1997*a*) gave results showing a significant correlation between antibody titre and infection intensity: in the latter instance it was both before and after chemotherapy. Furthermore, a several-fold discrepancy in circulating antigen concentrations has been found in 2 populations in Africa, in northern Senegal and eastern Zaire respectively, both of which are exposed to intense schistosomiasis transmission. Egg excretion patterns in the two patient groups were similar (van Lieshout, *et al*. 1998), but the Senegalese, who had the lower circulating antigen titres, had been exposed to transmission for a shorter period than the Zaireans. It was suggested that parasite fecundity was higher in the more recently exposed area (van Lieshout *et al*. 1998). If true, this may compromise the potential usefulness of worm-derived circulating antigens as indicators of intensity of pathology, most of which is acknowledged to be due to schistosome eggs (Warren, 1973).

A further problem that antibody detection has to overcome is an apparent lack of specificity. In epidemiological studies particularly, antibody is often found without concomitant parasitological evidence of infection (Doenhoff *et al*. 1993; Xue *et al*. 1993). This may be due to the method being indirect, with antibody remaining after self-cure or treatment of the infections. Antibody false-positivity may also be due to cross-reactivity between the antigens of human schistosome species and those of other infectious agents.

Set against the above possible explanations for lack of specificity are the arguments that: (1) schistosomes are long-lived and antibody false positives are found even in young age groups (Doenhoff *et al*. 1993) in which self-cure of infections is not so likely as when patients get older; (2) generally only a minority of samples from patients with other infections show a cross-reactivity to schistosome antigens (McLaren *et al*. 1981; Mott *et al*. 1987; Alves-Brito *et al*. 1992); (3) specificity of antibody detection tends to be lower in areas that are endemic for human schistosomiasis than in non-endemic areas (Doenhoff *et al*. 1993); and (4) the parasitological 'gold standard' methods of diagnosis, against which the degree of specificity of antibody detection is determined, are now acknowledged to be seriously lacking in sensitivity.

Unfortunately antigen detection methods do not appear to have sensitivity that is superior to that of standard parasitological methods: indeed, in populations with low prevalence and low intensity schistosomiasis, antigen detection appears to be even less sensitive than parasitology (van Leishout *et al*. 1995*a*; el Morshedy *et al*. 1996). Thus, for example, in a group of Dutch travellers with acute schistosomiasis serum titres of the circulating antigens CAA and CCA were 'generally very low' (van Lieshout *et al*. 1997). However, antigen detection in urine with an immunomagnetic bead immunoassay may allow sensitivity to be enhanced by allowing larger sample volumes to be processed (Nibbeling *et al*. 1997).

In some instances, therefore, the presence of specific anti-schistosome antibodies may provide the most sensitive indicator of the presence of infection. This is of course due to the fact that, in contrast to

https://doi.org/10.1017/S0031182099004205 Published online by Cambridge University Press

parasitological and antigen detection methods, antibody detection exploits the amplification of signal that is inherent in immune responsiveness.

Antibody detection may also provide the facility to distinguish between patent infections of different schistosome species. Thus, discrimination between *S. mansoni*, *S. haematobium* and/or *S. japonicum* infections can be improved by use of the relatively *S. mansoni*-specific CEF6 in ELISA (McLaren *et al.* 1981) and microsomal adult worm antigens of the different species in immunoblots (Tsang & Wilkins, 1997). This facility may not be readily available with antigen detection methods, since a single monoclonal antibody raised initially by immunization with a *S. mansoni* antigen was cross-reactive with the respective circulating antigens of several human and animal schistosome species (Agnew *et al.* 1995). Furthermore, human blood itself may contain glycoproteins that are immunologically cross-reactive with schistosome circulating antigens, such reactivity being reduced by treatment of serum with trichloracetic acid (de Jonge *et al.* 1987).

Cost is perhaps the factor with most influence on the choice of test, especially with respect to an infectious disease of people in economically less-developed countries. There will of course be a cost increase if 2 or more antibody detection tests are required, for example, in order to discriminate effectively between schistosome species. Set against this, there is a cost differential between antibody and antigen detection methods (in favour of the former) with regard to sample collection and preparation. Thus, while antibody assays can routinely be performed on blood samples which have been collected on filter paper (Doenhoff *et al.* 1993; Kamal *et al.* 1994), conventional filter papers cause a loss of sensitivity in antigen detection and specially formulated polypropylene fibre web may be required (Jamaly *et al.* 1997). Furthermore, blood or urine samples for antigen detection generally have to be pretreated with trichloroacetic acid or alkali before they can be used in the assay (de Jonge *et al.* 1987; Krijger *et al.* 1994), and antigen-detection requires production and application of an additional reagent in the form of the antigen-capturing (monoclonal) antibody.

CONCLUSION

In this review we have attempted to demonstrate that there is a wide range methods available for diagnosis of schistosomiasis by immunological means. Problems that are acknowledged to exist with parasitological diagnosis have been noted, and the relative advantages and disadvantages of parasitology, antigen detection and antibody detection have been considered. None of the methods considered above are without problems. There are as yet also no methods which can satisfactorily differentiate between infection and disease, or between forms of disease (e.g. hepatosplenic and intestinal schistosomiasis).

The objective of all efforts to control schistosomiasis is to reduce the prevalence and intensity of infection. Continuing success in that objective will only exacerbate already extant problems in current methods of diagnosis that stem from their inherently poor sensitivity (Doenhoff, 1998*c*). If it is confirmed that the apparent lack of specificity of antibody detection methods (relative to parasitology) is, in truth, a reflection of the former's greater sensitivity, antibody detection could increasingly become the diagnostic method of choice.

ACKNOWLEDGEMENTS

J.V.H. was supported by a BBSRC/CASE award. We are very grateful to Dr Peter Chiodini, Hospital for Tropical Diseases, London, for providing human infection sera for use in Fig. 2. Thanks also to Laura Layland for considerable help in the final stages of completing the manuscript.

REFERENCES

AGNEW, A., FULFORD, A. J. C., DE JONGE, N., KRIJGER, F. W., RODRIQUEZCHACON, M., GUTSMANN, V. & DEELDER, A. M. (1995). The relationship between worm burden and levels of a circulating antigen (CAA) of 5 species of *Schistosoma* in mice. *Parasitology* **111**, 67–76.

AGNEW, A. M., MURARE, H. M., SANDOVAL, S. N., DE JONGE, N., KRIJGER, F. W., DEELDER, A. M. & DOENHOFF, M. J. (1992). The susceptibility of adult schistosomes to immune attrition. *Memorias do Instituto Oswaldo Cruz* **87**, 87–93.

ALVES-BRITO, C. F., SIMPSON, A. J. G., BAHIA-OLIVEIRA, L. M. G., RABELLO, A. L. T., ROCHA, R. S., LAMBERTUCCI, J. R., GAZZINELLI, G., KATZ, N. & CORREA-OLIVEIRA, R. (1992). Analysis of anti-keyhole limpet haemocyanin antibody in Brazilians supports its use for the diagnosis of acute schistosomiasis mansoni. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 53–56.

AMBROISE-THOMAS, P. & ANDREWS, P. (1976). Development of fluorescent antibodies directed against larval stages, eggs, and adults of *Schistosoma mansoni* in mice harbouring unisexual or bisexual infections. *Tropenmedizin und Parasitologie* **27**, 483–488.

AURIAULT, C., GRAS-MASSE, H., PIERCE, R. J., BUTTERWORTH, A. E., WOLOWCZUK, I., CAPRON, M., OUMA, J. H., BALLOUL, J. M., KHALIFE, J., NEYRINCK, J. L., TARTAR, A., KOECH, D. & CAPRON, A. (1990). Antibody response of *Schistosoma mansoni*-infected human subjects to the recombinant P28 glutathione S-transferase and to synthetic peptides. *Journal of Clinical Microbiology* **28**, 1918–1924.

BARRETO, M. L., FRANCA SILVA, J. T., MOTT, K. E. & LEHMAN, J. S. (1978). Stability of faecal egg excretion in *Schistosoma mansoni* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **72**, 181–187.

BARRETO, M. L., SMITH, D. H. & SLEIGH, A. C. (1990). Implications of faecal egg count variation when using

the Kato-Katz method to assess *Schistosoma mansoni* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 554–555.

BELL, D. R. (1963). A new method for counting *Schistosoma mansoni* eggs in faeces. *Bulletin of the World Health Organisation* **29**, 525–530.

BERGGREN, W. L. & WELLER, T. H. (1967). Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **16**, 606–612.

BERGQUIST, N. R. (1992). *Immunodiagnostic Approaches in Schistosomiasis*. Chichester, John Wiley & Sons.

BERGWERFF, A. A., VAN DAM, G. J., ROTMANS, J. P., DEELDER, A. M., KAMERLING, J. P. & VLIEGENTHART, J. F. G. (1994). The immunologically reactive part of immunopurified circulating anodic antigen from *Schistosoma mansoni* is a threonine-linked polysaccharide consisting of → 6)-(beta-D-GlcpA-(1 → 3))-beta-D-GalpNAc-(1 → repeating units. *Journal of Biological Chemistry* **269**, 31510–31517.

BOCTOR, F. N., STEK, M. J., PETER, J. B. & KAMAL, R. (1987). Simplification and standardisation of dot-ELISA for human schistosomiasis mansoni. *Journal of Parasitology* **73**, 589–592.

BOGERS, J. J. P. M., NIBBELEING, H. A. M., VAN MARCK, E. A. E. & DEELDER, A. M. (1995). Immunoelectron microscopic localization of a circulating antigen in the excretory system of Schistosoma mansoni – ultra-structural-localization studies of the excretory system of *Schistosoma mansoni*. *Parasitology Research* **81**, 375–381.

BRAUN-MUNZINGER, R. A. (1986). Quantitative egg counts in schistosomiasis surveys. *Parasitology Today* **2**, 82–83.

CARLIER, Y., BOUT, D., BINA, J. C., CAMUS, D., FIGUEIREDO, J. F. M. & CAPRON, A. (1975). Immunological studies in human schistosomiasis. I. Parasitic antigen in urine. *American Journal of Tropical Medicine and Hygiene* **24**, 949–954.

CESARI, I. M., FERRER, A., KOMBILA, M., PICHARD, E., DECAM, C., LI-SHIU, Q., BOUT, D. & RICHARD-LENOBLE, D. (1998). Specificity of the solid phase alkaline phosphatase immunocapture assay for the diagnosis of human *Schistosoma mansoni* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 38–39.

CHAPPELL, C. L., DRESDEN, M. H., GRYSEELS, B. & DEELDER, A. M. (1990). Antibody response to *Schistosoma mansoni* adult worm cysteine proteinases in infected individuals. *American Journal of Tropical Medicine and Hygiene* **42**, 335–341.

CHEEVER, A. W. (1968). A quantitative post-mortem study of schistosomiasis mansoni in man. *American Journal of Tropical Medicine and Hygiene* **17**, 38–64.

CHI, F. & CARTER, C. E. (1990). Detection of a circulating antigen in human schistosomiasis japonica using a monoclonal antibody. *American Journal of Tropical Medicine and Hygiene* **42**, 347–351.

DAVERN, K. M., TIU, W. U., SAMARAS, N., GEARING, D. P., HALL, B. E., GARCIA, E. G. & MITCHELL, G. F. (1990). *Schistosoma japonicum* – monoclonal antibodies to the MR 26,000 schistosome glutathione S-transferase (Sj26) in an assay for circulating antigen in infected individuals. *Experimental Parasitology* **70**, 293–304.

DAY, J. H., GRANT, A. H., DOHERTY, J. F., CHIODINI, P. L. & WRIGHT, S. G. (1996). Schistosomiasis in travellers returning from sub-Saharan Africa. *British Medical Journal* **313**, 268–269.

DAZO, B. C. & BILES, J. E. (1974). Two new field techniques for detection and counting of *Schistosoma haematobium* eggs in urine samples, with an evaluation of both methods. *Bulletin of the World Health Organisation* **51**, 399–408.

DE CLERCQ, D., SACKO, M., VERCRUYSSE, J., DIARRA, A., LANDOURE, A., VAN DEN BUSSCHE, V., GRYSEELS, B. & DEELDER, A. (1995). Comparison of the circulating anodic antigen detection assay and urine filtration to diagnose *Schistosoma haematobium* infections in Mali. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **89**, 395–397.

DE CLERQ, D., SACKO, M., VERCRUYSSE, J., VAN DEN BUSSCHE, V., LANDOURE, A., DIARRA, A., GRYSEELS, B. & DEELDER, A. (1997). Assessment of cure by detection of circulating antigens in serum and urine, following schistosomiasis mass treatment in two villages of the office du Niger, Mali. *Acta Tropica* **68**, 339–346.

DEELDER, A. M., DE JONGE, N., BOERMAN, O. C., FILLIE, Y. E., HILBERATH, J., ROTMANS, J. P., GERRITSE, M. J. & SCHUT, D. W. O. (1989*a*). Sensitive determination of circulating anodic antigen in *Schistosoma mansoni* infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. *American Journal of Tropical Medicine and Hygiene* **40**, 268–272.

DEELDER, A. M., DE JONGE, N., FILLIE, Y. E., KORNELIS, D., HLAHA, D., QIAN, Z. L., DECALUWE, P. & POLDERMAN, A. M. (1989*b*). Quantitative determination of circulating antigens in human schistosomiasis mansoni using an indirect haemagglutination assay. *American Journal of Tropical Medicine and Hygiene* **40**, 50–54.

DEELDER, A. M., KLAPPE, H. T. M., VAN DEN AARDWEG, G. J. M. J. & VAN MEERBEKE, E. H. E. M. (1976). *Schistosoma mansoni*: demonstration of two circulating antigens in hamsters. *Experimental Parasitology* **40**, 189–197.

DEELDER, A. M. & KORNELIS, D. (1980). A comparison of the IFA and the ELISA for the demonstration of an antibody against schistosome gut-associated polysaccaride antigens in schistosomiasis. *Zietschrift für Parasitenkunde* **64**, 65–75.

DEELDER, A. M. & KORNELIS, D. (1981). Immunodiagnosis of recently acquired *Schistosoma mansoni* infection. A comparison of various immunological techniques. *Tropical and Geographical Medicine* **33**, 36–41.

DEELDER, A. M., KORNELIS, D., VAN MARCK, E. A. E., EVELEIGH, P. C. & VAN EGMOND, J. G. (1980). *Schistosoma mansoni*: characterization of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamster and human infections. *Experimental Parasitology* **50**, 16–32.

DEELDER, A. M., QIAN, Z. L., KREMSNER, P. G., ACOSTA, L., RABELLO, A. L. T., ENYONG, P., SIMARRO, P. P., VAN ETTEN, E. C. M., KRIJGER, F. W., ROTMANS, J. P., FILLIE, Y. E., DE JONGE, N., AGNEW, A. M. & VAN LIESHOUT, L. (1994). Quantitative diagnosis of *Schistosoma*

infections by measurement of circulating antigens in serum and urine. *Tropical and Geographic Medicine* **46**, 233–238.

DEELDER, A. M., RUITENBERG, E. J., KORNELIS, D. & STEERENBERG, P. A. (1977). *Schistosoma mansoni*: Comparison of the immunoperoxidase techniques DASS and ELISA, for human diagnosis. *Experimental Parasitology* **41**, 133–140.

DEELDER, A. M., VAN DAM, G. J., KORNELIS, D., FILLIE, Y. E. & VAN ZEYL, R. J. M. (1996). *Schistosoma* – analysis of monoclonal-antibodies reactive with the circulating antigens CAA and CCA. *Parasitology* **112**, 21–35.

DE JONGE, N., BOERMAN, O. C. & DEELDER, A. M. (1989). Time-resolved immunofluorometric assay (TR-IFMA) for the detection of schistosome circulating anodic antigen. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 659–663.

DE JONGE, N., FILLIE, Y. E. & DEELDER, A. M. (1987). A simple and rapid treatment (trichloracetic-acid precipitation) of serum samples to prevent non-specific reactions in the immunoassay of a proteoglycan. *Journal of Immunological Methods* **99**, 195–197.

DE JONGE, N., GRYSEELS, B., HILBERATH, G. W., POLDERMAN, A. M. & DEELDER, A. M. (1988). Detection of circulating anodic antigen by ELISA for seroepidemiology of schistosomiasis mansoni. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 591–594.

DE JONGE, N., RABELLO, A. L. T., KRIJGER, F. W., KREMSNER, P. G., ROCHA, R. S., KATZ, N. & DEELDER, A. M. (1991). Levels of the schistosome circulating anodic and cathodic antigens in serum of schistosomiasis patients from Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 756–759.

DE JONGE, N., SCHOMMER, G., KRIJGER, F. W., FELDMEIER, H., ZWINGENBERGER, K., STEINER, A., BIENZLE, U. & DEELDER, A. M. (1989). Presence of circulating anodic antigen in serum of *Schistosoma intercalatum*-infected patients from Gabon. *Acta Tropica* **46**, 115–120.

DENNIS, R. D., BAUMEISTER, S., LAUER, G., RICHTER, R. & GEYER, E. (1996). Neutral glycolipids of *Schistosoma mansoni* as feasible antigens in the detection of schistosomiasis. *Parasitology* **112**, 295–307.

DE NOYA, B. A., CESARI, I. M., LOSADA, S., COLMENARES, C., BALZAN, C., HOEBEKE, J. & NOYA, O. (1997). Evaluation of alkaline phosphatase immunoassay and comparison with other diagnostic methods in areas of low transmission of schistosomiasis. *Acta Tropica* **66**, 69–78.

DE VLAS, S. J. & GRYSEELS, B. (1992). Underestimation of *Schistosoma mansoni* prevalences. *Parasitology Today* **8**, 274–277.

DE VLAS, S. J., GRYSEELS, B., VAN OORTMARSSEN, G. J., POLDERMAN, A. M. & HABBEMA, J. D. F. (1993). A pocket chart to estimate true *Schistosoma mansoni* prevalences. *Parasitology Today* **9**, 305–307.

DISCH, J., GARCIA, M. M. A., KRIJGER, G. W., AMORIM, M. N., KATZ,N., DEELDER, A. M., GRYSEELS, B. & RABELLO, A. (1997). Daily fluctuations of levels of circulating cathodic antigen in urine of children infected with *Schistosoma mansoni* in Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 222–225.

DOENHOFF, M. J. (1998*a*). A role for granulomatous inflammation in the transmission of infectious disease: schistosomiasis and tuberculosis. *Parasitology* **115**, S113–S125.

DOENHOFF, M. J. (1998*b*). A vaccine for schistosomiasis: alternative approaches. *Parasitology Today* **14**, 105–109.

DOENHOFF, M. J. (1998*c*). Is schistosomicidal chemotherapy sub-curative? Implications for drug resistance. *Parasitology Today* **14**, 434–435.

DOENHOFF, M. J., BUTTERWORTH, A. E., HAYES, R. J., STURROCK, R. F., OUMA, J. H., KOECH, D., PRENTICE, M. & BAIN, J. (1993). Seroepidemiology and serodiagnosis of schistosomiasis in Kenya using crude and purified egg antigens of *Schistosoma mansoni* in ELISA. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 42–48.

DOENHOFF, M. J., DUNNE, D. W. & LILLYWHITE, J. E. (1989). Serology of *Schistosoma mansoni* infections after chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 237–238.

DUNNE, D. W., AGNEW, A. M., MODHA, J. & DOENHOFF, M. J. (1986). *Schistosoma mansoni* egg antigens: preparation of rabbit antisera with monospecific immunoprecipitating activity, and their use in antigen characterization. *Parasite Immunology* **8**, 575–586.

DUNNE, D. W., BAIN, J., LILLYWHITE, J. & DOENHOFF, M. J. (1984). The stage-, strain-, and species-specificity of a *Schistosoma mansoni* egg antigen fraction with serodiagnostic potential. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 460–470.

DUNNE, D. W., HILLYER, G. V. & VAZQUEZ, G. (1988). *Schistosoma mansoni* egg antigens (CEF6): immunoserology with oxaminiquine-treated patients and involvement of CEF6 in the circumoval precipitin reaction. *American Journal of Tropical Medicine and Hygiene* **38**, 508–514.

DUNNE, D. W., JONES, F. M. & DOENHOFF, M. J. (1991). The purification, characterization, serological activity and hepatotoxic properties of two cationic glycoproteins (alpha-1 and omega-1) from *Schistosoma mansoni* eggs. *Parasitology* **103**, 225–236.

DUNNE, D. W., LUCAS, S., BICKLE, Q., PEARSON, S., MADGWICK, L., BAIN, J. & DOENHOFF, M. J. (1981). Identification and partial purification of an antigen (omega 1) from *Schistosoma mansoni* eggs which is putatively hepatotoxic in T-cell deprived mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 54–71.

EBRAHIM, A., EL MORSHEDY, H., OMER, E., EL DALY, S. & BARAKAT, R. (1997). Evaluation of the Kato-Katz thick smear and formol ether sedimentation techniques for quantitative diagnosis of *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene* **57**, 706–708.

EL MORSHEDY, H., KINOSIEN, B., BARAKAT, R., OMER, E., KHAMIS, N., DEELDER, A. M. & PHILLIPS, M. (1996). Circulating anodic antigen for detection of *Schistosoma mansoni* infection in Egyptian patients. *American Journal of Tropical Medicine and Hygiene* **54**, 149–153.

EL MISSIRY, A. G., EL SEROUGI, A. O., SALAMA, M. M. I. & KAMAL, A. M. (1990). Evaluation of the dot ELISA

technique in the serodiagnosis of schistosomiasis in Egypt. *Journal of the Egyptian Society of Parasitology* **20**, 639–645.

EL SAYED, L. H., GHONEIM, H., DEMIAN, S. R., EL SAYED, M. H., TAWFIK, N. M., SAKR, I., ABOU-BASHA, L., RENGANATHAN, E., KLINKERT, M. Q. & ABOURAWASH, N. (1998). Diagnostic significance of *Schistosoma mansoni* proteins Sm31 and Sm32 in human schistosomiasis in an endemic area in Egypt. *Tropical Medicine and International Health* **3**, 721–727.

ELTIRO, F., YE-EBIYO, Y. & TAYLOR, M. G. (1992). Evaluation of an enzyme linked immunosorbent assay (ELISA) using *Schistosoma mansoni* soluble egg antigen as a diagnostic tool for *Schistosoma mansoni* infection in Ethiopian schoolchildren. *Journal of Tropical Medicine and Hygiene* **95**, 52–56.

ENGELS, D., SINZINKAYO, E., DE VLAS, S. J. & GRYSEELS, B. (1997). Intraspecimen fecal egg count variation in *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene* **57**, 571–577.

ENGELS, D., SINZINKAYO, E. & GRYSEELS, B. (1996). Day-to-day egg count fluctuation in *Schistosoma mansoni* infection and its operational implications. *American Journal of Tropical Medicine and Hygiene* **54**, 319–324.

ENGVALL, E. & PERLMANN, P. (1971). ELISA: quantitative assay of immunoglobulin G. *Immunochemistry* **8**, 871–874.

EVENGARD, B. (1990). Diagnostic and clinical aspects of schistosomiasis in 182 patients treated on a Swedish ward for tropical diseases during a 10 year period. *Scandinavian Journal of Infectious Diseases* **22**, 585–591.

EVENGARD, B., HAMMARSTROM, L., SMITH, C. I. & LINDER, E. (1990). Early antibody responses in human schistosomiasis. *Clinical and Experimental Immunology* **80**, 69–76.

EVENGARD, B., WOLOWCZUK, I., MARGUERITE, M., HAMMARSTROM, L., SMITH, E. & AURIAULT, C. (1994). IgG subclass-associated differences in anti-schistosomal antibody specificity. *Scandinavian Journal of Immunology* **40**, 618–622.

FEINSTEIN, A. R. (1975). Clinical biostatistics. XXXI: On the sensitivity, specificity and discrimination of diagnostic tests. *Clinical and Pharmacological Therapeutics* **17**, 104–116.

FELDMEIER, H. & POGGENSEE, G. (1993). Diagnostic techniques in schistosomiasis control. A review. *Acta Tropica* **52**, 205–220.

FERRARI, T. C. A., MOREIRA, P. R. R., OLIVEIRA, R. C., FERRARI, M. L. A., GAZZINELLI, G. & CUNHA, A. S. (1995). The value of an enzyme-linked immunosorbent assay for the diagnosis of schistosomiasis mansoni myeloradiculopathy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 496–500.

FLETCHER, R. H., FLETCHER, A. W. & WAGNER, E. H. (1996). *Clinical Epidemiology : The Essentials*. (3rd Ed) Baltimore, Williams & Wilkins.

GAZZINELLI, G., LAMBERTUCCI, J. R., KATZ, N., ROCHA, R. S., LIMA, M. S., & COLLEY, D. G. (1985). Immune responses during human schistosomiasis mansoni. XI: Immunologic status of patients with acute infections and after treatment. *Journal of Immunology* **135**, 2121–2127.

GHANDOUR, A. M., TRICKER, K., DOENHOFF, M. J., ALROBAI, A. A. & BANAJA, A. A. (1997). An enzyme-linked immunosorbent assay using *Schistosoma mansoni* purified egg antigen for the diagnosis of schistosomiasis in Saudi Arabia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 287–289.

GROGAN, J. L., KREMSNER, P. G., VAN DAM, G. J., DEELDER, A. M. & YAZDANBAKSH, M. (1997*a*). Anti-schistosome IgG4 and IgG at 2 years after chemotherapy: infected versus uninfected individuals. *Journal of Infectious Diseases* **176**, 1344–1350.

GROGAN, J. L., KREMSNER, P. G., VAN DAM, G. J., METZGER, W., MORDMULLER, B., DEELDER, A. M. & YAZDANBAKSH, M. (1996). Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. *Journal of Infectious Diseases* **173**, 1242–1247.

GROGAN, J., ROTMANS, P., GHONHEIM, H., DEELDER, A. M., YAZDANBAKSH, M. & KLINKERT, M. Q. (1997*b*). Recognition of recombinant *Schistosoma mansoni* cathepsins B and L by human IgG1 and IgG4 antibodies. *Parasite Immunology* **19**, 215–220.

GRYSEELS, B., STELMA, F. F., TALLA, I., VAN DAM, G. J., POLMAN, K., SOW, S., DIAW, M., STURROCK, R. F., DOEHRINGSCHWERDTFEGER, E., KARDORFF, R., DECAM, C., NIANG, M. & DEELDER, A. M. (1994). Epidemiology, immunology and chemotherapy of *Schistosoma mansoni* infections in a recently exposed community in Senegal. *Tropical and Geographical Medicine* **46**, 209–219.

GRYSEELS, B. & DE VLAS, S. J. (1996). Worm burdens in schistosome infections. *Parasitology Today* **12**, 115–119.

GRZYCH, J.-M., DISSOUS, C., CAPRON, M., TORRES, S., LAMBERT, P.-H. & CAPRON, A. (1987). *Schistosoma mansoni* shares a protective carbohydrate epitope with keyhole limpet haemocyanin. *Journal of Experimental Medicine* **165**, 865–878.

GUNDERSON, S. G., HAAGENSEN, I., JONASSEN, T. O., FIGENSCHAU, K. J., DE JONGE, N. & DEELDER, A. M. (1992). Magnetic bead antigen capture enzyme-linked immunoassay in microtitre trays for rapid detection of schistosomal circulating anodic antigen. *Journal of Immunological Methods* **148**, 1–8.

HAMBURGER, J., PELLEY, R. P. & WARREN, K. S. (1976). *Schistosoma mansoni* soluble egg antigens: determination of the stage and species specificity of their serologic reactivity by radioimmunoassay. *Journal of Immunology* **117**, 1561–1566.

HAMILTON, J. V., CHIODINI, P. L., FALLON, P. G. & DOENHOFF, M. J. (1999). Periodate-sensitive immunological cross-reactivity between keyhole limpet haemocyanin (KLH) and serodiagnostic *Schistosoma mansoni* egg antigens. *Parasitology* **118**, 83–89.

HANCOCK, K., MOHAMED, Y. B., XUE, H. C., NOH, J., DOTSON, E. M. & TSANG, V. C. W. (1997). A recombinant protein from *Schistosoma mansoni* useful for the detection of *S. mansoni* and *Schistosoma haematobium* antibodies. *Journal of Parasitology* **83**, 612–618.

HANCOCK, K. & TSANG, V. C. W. (1986). Development and optimisation of the FAST-ELISA for detecting

antibodies to *Schistosoma mansoni*. *Journal of Immunological Methods* **92**, 167–176.

HASSAN, F., ABDEL-WAHAB, M. F., NOSSEUR, A., SEDEEKI, S., SHEHATA, A. & MASOOD, M. A. (1979). Evaluation of the enzyme-linked immunosorbent assay in the immunodiagnosis of schistosomiasis. *Journal of Tropical Medicine and Hygiene* **82**, 3–7.

HASSAN, M. M., BADAWI, M. A. & STRAND, M. (1992). Circulating schistosomal antigen in diagnosis and assessment of cure in individuals infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **46**, 737–744.

HILLYER, G. V. & PELLEY, R. P. (1980). The major serological antigen (MSA1) from *Schistosoma mansoni* eggs is a circumoval precipitin. *American Journal of Tropical Medicine and Hygiene* **29**, 582–585.

HULDT, G., LAGERQUIST, B., PHILLIPS, T., DRAPER, C. C. & VOLLER, A. (1975). Detection of antibodies in schistosomiasis by enzyme-linked immunosorbent assay (ELISA). *Annals of Tropical Medicine and Parasitology* **69**, 483–488.

IDRIS, M. A. & RUPPEL, A. (1988). Diagnostic Mr 31/32000 *Schistosoma mansoni* proteins (Sm31/32): Reaction with sera from Sudanese patients infected with *S. mansoni* or *S. haematobium*. *Journal of Helminthology* **62**, 95–101.

ISMAIL, M. M., BRUCE, J. I., ATTIA, M., TAYEL, S. E., SABAH, A. A. & EL-AHMEDAWY, B. A. (1989). The detection of IgE by radio-allergosorbent technique (RAST) and ELISA in Egyptian cases of schistosomiasis. *Journal of the Egyptian Society of Parasitology* **19**, 29–34.

JAMALY, S., CHIHANI, T., DEELDER, A. M., GABONE, R., NILSSON, L. A. & OUCHTERLONY, O. (1997). Polypropylene fibre web, a new matrix for sampling blood for immunodiagnosis of schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 412–415.

JEMANEH, L. (1993). Comparison of immunodiagnosis (ELISA) and stool examination (Kato technique) in the diagnosis of *Schistosoma mansoni* in Ethiopia. *Ethiopian Medical Journal* **31**, 37–49.

KAGAN, I. (1968). A critical review of immunological methods for the diagnosis of Bilharziasis. *Bulletin of the World Health Organisation* **25**, 611–674.

KAMAL, K. A., SHAHEEN, H. I. & EL SAID, A. A. (1994). Applicability of ELISA on buffer-eluates of capillary blood spotted on filter papers for the diagnosis and clinical staging of human schistosomiasis. *Tropical and Geographical Medicine* **46**, 138–141.

KARANJA, D. M. S., COLLEY, D. G., NAHLEN, B. L., OUMA, J. H. & SECOR, W. E. (1997). Studies on schistosomiasis in Western Kenya. I. Evidence for immune facilitated excretions of schistosome eggs from patients with *Schistosoma mansoni* and Human ImmunodefficiencyVirus co-infections. *American Journal of Tropical Medicine and Hygiene* **56**, 515–521.

KATO, K. & MIURA, N. (1954). Comparative examinations. *Japanese Journal of Parasitology* **3**, 35–37.

KATZ, N., CHAVES, A. & PELLEGRINO, J. (1972). A simple device for quantitative stool thick smear technique in schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical Sao Paulo* **14**, 397–400.

KHALIL, H. M., MAKLED, M. K. I., EL-MISSIRY, A. G., KHALIL, N. M. & SONBOL, S. E. (1989). The application of *Schistosoma mansoni* adult and soluble egg antigens for the serodiagnosis of schistosomiasis by CIEP, IHA and ELISA. *Journal of the Egyptian Society of Parasitology* **19**, Suppl. 2, 827–843.

KLINKERT, M. Q., BOMMERT, K., MOSER, D., FELLEISEN, R., LINK, G., DOUMBO, O. & BECK, E. (1991). Immunological analysis of cloned schistosomiasis mansoni antigens Sm31 and Sm32 with sera of schistosomiasis patients. *Tropical and Medical Parasitology* **42**, 319–324.

KLINKERT, M. Q., RUPPEL, A. & BECK, E. (1987). Cloning of diagnostic 31/32 kDa antigens of *Schistosoma mansoni*. *Molecular and Biochemical Parasitology* **25**, 247–255.

KNIGHT, W. B., HIATT, R. A., CLINE, B. L. & RITCHIE, L. S. (1976). A modification of the formol-ether concentration technique for increased sensitivity in detecting *Schistosoma mansoni* eggs. *American Journal of Tropical Medicine and Hygiene* **25**, 818–823.

KRIJGER, F. W., VAN LIESHOUT, L. & DEELDER, A. M. (1994). A simple technique to pretreat urine and serum samples for quantitation of schistosome circulating anodic and cathodic antigen. *Acta Tropica* **56**, 55–63.

LANGLEY, G., KARIUKI, H. C., HAMMERSLEY, A. P., OUMA, J. H., BUTTERWORTH, A. E. & DUNNE, D. W. (1994). Human IgG subclass responses and subclass restriction to *Schistosoma mansoni* egg antigens. *Immunology* **83**, 651–658.

LI, Y. L., IDRIS, M. A., CORACHAN, M., HAN, J. J., KIRSCHIFINK, M. & RUPPEL, A. (1996). Circulating antigens in schistosomiasis: detection of 31/32-kDa proteins in sera from patients infected with *Schistosoma japonicum*, *S. mansoni*, *S. haematobium*, or *S. intercalatum*. *Parasitology Research* **82**, 14–18.

LI, Y. L., SONG, W. J., HAN, J. J. & RUPPEL, A. (1994). Detection of *Schistosoma japonicum* antigen (Sj31/32) in sera of Chinese patients using a sandwich ELISA based on a monoclonal antibody. *Tropical Medicine and Parasitology* **45**, 115–118.

LI, Y. S., ROSS, A. G. P., LI, Y., HE, Y. K., LUO, X. S. & MCMANUS, D. P. (1997). Serological diagnosis of *Schistosoma japonicum* infections in China. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 19–21.

LI, Z., KING, C. L., OGUNDIPE, J. O., LICATE, L. S. & BLANTON, R. E. (1995). Preferential recognition by human IgE and IgG4 of a species specific *Schistosoma haematobium* serine protease inhibitor. *Journal of Infectious Diseases* **171**, 416–422.

LIPPS, G., FULLKRUG, R. & BECK, E. (1996). Cathepsin-B of *Schistosoma mansoni* – purification and activation of the recombinant proenzyme by *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **271**, 1717–1725.

LIVINGSTON, P. O. (1995). Approaches to augmenting the immunogenicity of melanoma gangliosides: from whole melanoma cells to ganglioside-KLH conjugate vaccines. *Immunological Reviews* **145**, 147–166.

LUNDE, M. N. & OTTESEN, E. A. (1980). Enzyme-linked immunosorbent assay (ELISA) for detecting IgM and IgE antibodies in human schistosomiasis. *American Journal of Tropical Medicine and Hygiene* **29**, 82–85.

LUNDE, M. N., OTTESEN, E. A. & CHEEVER, A. W. (1979). Serological differences between acute and chronic

schistosomiasis mansoni detected by enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene* **28**, 87–91.

MADDISON, S. E. (1987). The present status of serodiagnosis and seroepidemiology of schistosomiasis. *Diagnostic Microbiology and Infectious Disease* **7**, 93–105.

MADDISON, S. E. (1991). Serodiagnosis of parasitic diseases. *Clinical Microbiology Reviews* **4**, 457–469.

MADDISON, S. E., SLEMENDA, S. B., TSANG, V. C. & POLLARD, R. A. (1985). Serodiagnosis of *Schistosoma mansoni* with adult worm antigen in an enzyme-linked immunosorbent assay using a standard curve developed with a reference serum pool. *American Journal of Tropical Medicine and Hygiene* **34**, 484–494.

MANSOUR, M. M., OMER-ALI, P., FARID, Z., SIMPSON, A. J. G. & WOODY, J. (1989). Serological differentiation of acute and chronic schistosomiasis mansoni by antibody responses to keyhole limpet haemocyanin. *American Journal of Tropical Medicine and Hygiene* **41**, 338–344.

MARTIN, L. K. & BEAVER, P. C. (1968). Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. *American Journal of Tropical Medicine and Hygiene* **17**, 382–391.

MARKL, J., NOUR EL DIN, M., WINTER-SIMANOWSKI, S. & SIMANOWSKI, U. A. (1991). Specific IgG activity of sera from Egyptian schistosomiasis patients to keyhole limpet haemocyanin (KLH). *Naturwissenschaften* **78**, 30–31.

MCLAREN, M. L., DRAPER, C. C., ROBERTS, J. M., MINTER-GOEDBLOED, E., LIGHTHART, G. S., TEESDALE, C. H., AMIN, M. A., OMER, A. H. S., BARTLETT, A. & VOLLER, A. (1978). Studies on the enzyme linked immunosorbent assay (ELISA) test for *Schistosoma mansoni* infections. *Annals of Tropical Medical Parasitology* **72**, 243–253.

MCLAREN, M. L., LILLYWHITE, J. E., DUNNE, D. W. & DOENHOFF, M. J. (1981). Serodiagnosis of human *Schistosoma mansoni* infections: enhanced sensitivity and specificity in egg antigen omega1 and alpha1. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 72–79.

MERKELBACH, A., HASSE, S., DELL, R., ESCHBECK, A. & RUPPEL, A. (1994). cDNA sequences of *Schistosoma japonicum* coding for two cathepsin-like proteins and Sj32. *Tropical Medicine and Parasitology* **45**, 193–198.

MOSER, D., DOENHOFF, M. J. & KLINKERT, M. Q. (1992). A stage-specific calcium-binding protein expressed in eggs of *Schistosoma mansoni*. *Molecular and Biochemical Parasitology* **51**, 229–238.

MOSER, D., DOUMBO, O. & KLINKERT,M.-Q. (1990). The humoral response to heat shock protein 70 in human and murine schistosomiasis mansoni. *Parasite Immunology* **12**, 341–342.

MOTT, K. E. & DIXON, H. (1982). Collaborative study on antigens for immunodiagnosis of schistosomiasis. *Bulletin of the World Health Organisation* **60**, 233–244.

MOTT, K. E., DIXON, H., CARTER, C. E., GARCIA, E., ISHII, A., MATSUDA, H., MITCHELL, G., OWHASHI, M., TANAKA, H. & TSANG, V. C. (1987). Collaborative study on antigens for the immunodiagnosis of *Schistosoma japonicum* infection. *Bulletin of the World Health Organisation* **65**, 233–244.

NASH, T. E., GARCIACOYCO, C., RUIZTIBEN, E., NAZARIOLOPEZ, H. A., VAZQUEZ, G. & TORRESBORGES, A. (1983). Differentiation of acute and chronic schistosomiasis by antibody responses to specific schistosome antigens. *American Journal of Tropical Medicine and Hygiene* **32**, 776–784.

NASH, T. E., LUNDE, M. N. & CHEEVER, A. W. (1981). Analysis and antigenic activity of a carbohydrate fraction derived from adult *Schistosoma mansoni*. *Journal of Immunology* **126**, 805–810.

NASH, T. E., PRESCOTT, B. & NEVA, F. A. (1974). The characteristics of a circulating antigen in schistosomiasis. *Journal of Immunology* **112**, 1500–1507.

NEWPORT, G. R., MCKERROW, J. H., HEDSTROM, R. C., KALLESTAD, J., TARR, P., KLEBANOFF, S. & AGABIAN, N. (1988). Identification, molecular cloning and expression of a schistosome antigen displaying diagnostic potential. *American Journal of Tropical Medicine and Hygiene* **38**, 540–546.

NIBBELING, H. A. M., VAN ETTEN, L., FILLIE, Y. E. & DEELDER, A. M. (1997). Enhanced detection of *Schistosoma* circulating antigens by testing 1 ml urine samples using immunomagnetic beads. *Acta Tropica* **66**, 85–92.

OKABE, K. & TANAKA, T. (1961). Urine precipitation reaction for schistosomiasis japonica. *Kurume Medical Journal* **8**, 24–29.

PELLEY, R. P, WARREN, K. S. & JORDAN, P. (1977). Purified antigen radioimmunoassay in serological diagnosis of schistosomiasis mansoni. *Lancet* **ii**, 781–785.

PETERS, P. A., MAHMOUD, A. A. F., WARREN, K. S., OUMAN, J. H. & ARAP SIONGOK, T. K. (1976). Field studies of a rapid, accurate means of quantifying *Schistosoma haematobium* eggs in urine samples. *Bulletin of the World Health Organisation* **54**, 159–162.

PINO-HEISS, S., PETITT, M., BECKSTEAD, J. H. & MCKERROW, J. H. (1986). Preparation of monoclonal antibodies and evidence for a host immune response to the preacetabular gland proteinase of *Schistosoma mansoni* cercariae. *American Journal of Tropical Medicine and Hygiene* **35**, 536–543.

PUJOL, F. H. & CESARI, I. M. (1990). Antigenicity of adult *S. mansoni* alkaline phosphatase. *Parasite Immunology* **12**, 189–198.

PUJOL, F. H., DE NOYA, B. A. & CESARI, I. M. (1989). Immunodiagnosis of schistosomiasis mansoni with APIA (alkaline phosphatase immunoassay). *Immunological Investigations* **18**, 1071–1080.

RABELLO, A. (1997). Diagnosing schistosomiasis. *Memorias do Instituto Oswaldo Cruz* **92**, 669–676.

RABELLO, A. L. T., GARCIA, M. M. A., DA SILVA, R. A. P., ROCHA, R. S. & KATZ, N. (1997). Humoral immune responses in patients with acute *Schistosoma mansoni* infection who were followed up two years after treatment. *Clinical Infectious Diseases* **24**, 304–308.

RABELLO, A. L. T., GARCIA, M. M. A., NETO, E. D., ROCHA, R. S. & KATZ, N. (1993). Dot-dye-immunoassay and dot-ELISA for the serological differentiation of acute and chronic schistosomiasis-mansoni using keyhole limpet haemocyanin as antigen. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 279–281.

RABELLO, A. L. T., NETO, E. D., GARCIA, M. M. A. & KATZ, N. (1992). Dot-dye immunoassay for the diagnosis of schistosomiasis mansoni. *Memorias do Instituto Oswaldo Cruz* **87**, 187–190.

RAMZY, R. M. R., FARIS, R., BAHGAT, M., HELMY, H., FRANKLIN, C. & McKERROW, J. H. (1997). Evaluation of a stage-specific proteolytic enzyme of *Schistosoma mansoni* as a marker of exposure. *American Journal of Tropical Medicine and Hygiene* **56**, 668–673.

RIDLEY, D. S. & HAWGOOD, B. C. (1956). The value of formal ether concentration of fecal cysts and ova. *Journal of Clinical Pathology* **9**, 74–76.

RUIZ-TIBEN, E., HILLYER, G. V., KNIGHT, W. B., GOMEZ DE RIOS, I. & WOODALL, J. P. (1979). Intensity of infection with *Schistosoma mansoni*: its relationship to the sensitivity and specificity of serologic tests. *American Journal of Tropical Medicine and Hygiene* **28**, 230–236.

RUPPEL, A., DIESFELD, H. J. & ROTHER, U. (1985). Immunoblot analysis of *Schistosoma mansoni* antigens with sera of schistosomiasis patients: diagnostic potential of an adult schistosome polypeptide. *Clinical and Experimental Immunology* **62**, 499–506.

RUPPEL, A., IDRIS, M. A., SULAIMAN, S. M. & HILALI, A. M. H. (1990). *Schistosoma mansoni* diagnostic antigens (Sm31/32): a sero-epidemiological study in the Sudan. *Tropical Medicine and Parasitology* **41**, 127–130.

RUPPEL, A., SHI, Y. E., WEI, D. X. & DIESFELD, H. J. (1987). Sera of *Schistosoma japonicum* infected patients cross-react with diagnostic 31/32 kDa proteins of *Schistosoma mansoni*. *Clinical and Experimental Immunology* **69**, 291–298.

SANTORO, F. Y., CARLIER, Y., BOROJEVIC, R., BOUT, D., TACHON, P. & CAPRON, A. (1977). Parasite M antigen in milk from mothers infected with *Schistosoma mansoni*. *Annals of Tropical Medicine and Parasitology* **71**, 121–123.

SATTI, M. Z., LIND, P., VENNERVALD, B. J., SULAIMAN, S. M., DAFFALLA, A. A. & GHALIB, H. W. (1996*a*). Specific immunoglobulin in measurements related to exposure and resistance to *Schistosoma mansoni* infection in Sudanese canal cleaners. *Clinical and Experimental Immunology* **106**, 45–54.

SATTI, M. Z., EBBESEN, F., VENNERVALD, B., LIND, P., GHALIB, H., SULAIMAN, S., DAFFALLA, A. & SKOV, P. S. (1996*b*). Use of a new glass microfibre histamine-release method to study the modulation of the host response in human schistosomiasis mansoni – individuals with different degrees of exposure to the disease show differing antibody biological function. *Tropical Medicine and International Health* **1**, 655–666.

SLEIGH, A., HOFF, R., MOTT, K., BARRETO, M., DE PAIVA, T. M., PEDROSA, J. D. S. & SHERLOCK, I. (1982). Comparison of filtration staining (Bell) and thick smear (Kato) for the detection and quantitation of *Schistosoma mansoni* eggs in faeces. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 403–406.

TANABE, M., OKAZAKI, M., KOBAYASHI, S., KANEKO, N., SEKIGUCHI, T., TATENO, S., MOTTA, S. R. N. & TAKEUCHI, T. (1990). Serological studies on schistosomiasis mansoni in the Northeast Brazil. *Revista do Instituto de Medicina Tropical Sao Paulo* **32**, 121–131.

TOSSWILL, J. H. C. & RIDLEY, D. S. (1986). An evaluation of the ELISA for schistosomiasis in a hospital population. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 435–441.

TOY, L., PETIT, M., WANG, Y. F., HEDSTROM, R. & McKERROW, J. H. (1987). The immune response to stage-specific proteolytic enzymes of *Schistosoma mansoni*. In *Molecular Paradigms for Eradicating Helminthic Parasites* (ed. Agabian, N., Goddman, H. & Nogueira, N.) pp. 85–103. Alan R. Liss Inc., New York, USA.

TSANG, V. C. W., HANCOCK, K., KELLY, M. A., WILSON, B. C. & MADDISON, S. E. (1983*a*). *Schistosoma mansoni* adult microsomal antigens, a serologic reagent. 2. Specificity of antibody responses to the *S. mansoni* microsomal antigen. *Journal of Immunology* **130**, 1366–1370.

TSANG, V. C. W., TAO, Y. X., QUI, L. S. & XUE, H. C. (1982). Fractionation and quantitation of egg antigens from *Schistosoma japonicum* by the single-tube kinetic-dependent enzyme-linked immunosorbent-assay (k-ELISA) – higher antigenic activity in urea-soluble than in aqueous-soluble fractions. *Journal of Parasitology* **68**, 1034–1043.

TSANG, V. C. W., TSANG, K. R., HANCOCK, K., KELLY, M. A., WILSON, B. C. & MADDISON, S. E. (1983*b*). *Schistosoma mansoni* adult microsomal antigens, a serologic reagent. 1. Systematic fractionation, quantitation, and characterization of antigenic components. *Journal of Immunology* **130**, 1359–1365.

TSANG, V. C. W. & WILKINS, P. P. (1997). Immunodiagnosis of schistosomiasis. *Immunological Investigations* **26**, 175–188.

VALLI, L. C. P., KANAMURA, H. Y., DA SILVA, R. M., SILVA, M. I. P. G., VELLOSA, S. A. G. & GARCIA, E. T. (1997). Efficacy of an enzyme-linked immunosorbent assay in the diagnosis of and serologic distinction between acute and chronic *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene* **57**, 358–362.

VAN DAM, G. J., BERGWERF, A. A., THOMASOATES, J. E., ROTMANS, J. P., KAMERLING, J. P., FLIEGENTHART, J. F. G. & DEELDER, A. M. (1994). The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis-X as repeating unit. *European Journal of Biochemistry* **225**, 467–482.

VAN DAM, G. J., CLAAS, F. H. J., YAZDANBAKHSH, M., KRUIZE, Y. C. M., VAN KEULEN, A. C. I., FERREIRA, S. T. M. F., ROTMANS, J. P. & DEELDER, A. M. (1996). *Schistosomas mansoni* excretory circulating cathodic antigen shares Lewis-x epitopes with a human granulocyte surface antigen and evokes host antibodies mediating complement-dependent lysis of granulocytes. *Blood* **88**, 4246–4251.

VAN DAM, G. J., STELMA, F. F., GRYSEELS, B., FERREIRA, S. T. M. F., TALLA, I., NIANG, M., ROTMANS, J. P. & DEELDER, A. M. (1996). Antibody response patterns against *Schistosoma mansoni* in a recently exposed community in Senegal. *Journal of Infectious Diseases* **173**, 1232–1241.

VAN ETTEN, L., ENGELS, D., KRIJGER, F. W., NKULIKYINKA, L., GRYSEELS, B. & DEELDER, A. M. (1996). Fluctuation of schistosome circulating antigen levels in urine of individuals with *Schistosoma mansoni* infection in Burundi. *American Journal of Tropical Medicine and Hygiene* **54**, 348–351.

VAN ETTEN, L., KREMSNER, P. G., KRIJGER, F. W. &

DEELDER, A. M. (1997*a*). Day-to-day variation of egg output and schistosome circulating antigens in urine of *Schistosoma haematobium*-infected school children from Gabon and follow-up after chemotherapy. *American Journal of Tropical Medicine and Hygiene* **57**, 337–341.

VAN ETTEN, L., VAN LIESHOUT, L., MANSOUR, M. M. & DEELDER, A. M. (1997*b*). A reagent strip antigen capture assay for the assessment of cure of schistosomiasis patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 154–155.

VAN LIESHOUT, L., DE JONGE, N., EL MASRY, N. A., MANSOUR, M. M., BASSILY, S., KRIJGER, F. W. & DEELDER, A. M. (1994). Monitoring the efficacy of different doses of praziquantel by quantification of circulating antigens in serum and urine of schistosomiasis patients. *Parasitology* **108**, 519–526.

VAN LIESHOUT, L., DE JONGE, N., EL MASRY, N. A., MANSOUR, M. M., KRIJGER, F. W. & DEELDER, A. M. (1992). Improved diagnostic performance of the circulating antigen assay in human schistosomiasis by parallel testing for circulating anodic and cathodic antigens in serum and urine. *American Journal of Tropical Medicine and Hygiene* **47**, 463–469.

VAN LIESHOUT, L., PANDAY, U. G., DE JONGE, N., KRIJGER, F. W., OOSTBURG, B. F. J., POLDERMAN, A. M. & DEELDER, A. M. (1995*a*). Immunodiagnosis of schistosomiasis mansoni in a low endemic area in Surinam by determination of the circulating antigens CAA and CCA. *Acta Tropica* **59**, 19–29.

VAN LIESHOUT, L., POLDERMAN, A. M., DE VLAS, S. J., DE CALUWE, P., KRIJGER, F. W., GRYSEELS, B. & DEELDER, A. M. (1995*b*). Analysis of worm burden variation in human *Schistosoma mansoni* infections by determination of serum levels of circulating anodic antigen and circulating cathodic antigen. *Journal of Infectious Diseases* **172**, 1336–1342.

VAN LIESHOUT, L., POLDERMAN, A. M., VISSER, L. G., VERWAY, J. J. & DEELDER, A. M. (1997). Detection of the circulating antigens CAA and CCA in a group of Dutch travellers with acute schistosomiasis. *Tropical Medicine and International Health* **2**, 551–557.

VAN LIESHOUT, L., POLMAN, K., GRYSEELS, B. & DEELDER, A. M. (1998). Circulating anodic antigen levels in two areas endemic for schistosomiasis mansoni indicate differences in worm fecundity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 115–119.

VAN'T WOUT, A. B., DE JONGE, N., WOOD, S. M., VAN LIESHOUT, L., MITCHELL, G. F. & DEELDER, A. M. (1995). Serum levels of circulating anodic antigen and circulating cathodic antigen detected in mice infected with *Schistosoma japonicum* or *S. mansoni*. *Parasitology Research* **81**, 434–437.

VENKATESAN, P. & WAKELIN, D. (1993). ELISAs for parasitologists: or lies, damned lies and ELISAs. *Parasitology Today* **9**, 228–232.

VERWEIJ, J. J., POLDERMAN, A. M., VISSER, L. G. & DEELDER, A. M. (1995). Measurement of antibody response to keyhole limpet haemocyanin was not adequate for early diagnosis of schistosomiasis in a group of Dutch visitors to Mali. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 48–50.

VISSER, L. G., POLDERMAN, A. M. & STUIVER, P. C. (1995). Outbreak of schistosomiasis among travellers returning from Mali, West Africa. *Clinical Infectious Diseases* **20**, 280–285.

VOLLER, A., BARTLETT, A. & BIDWELL, D. E. (1976). Enzyme immunoassays for parasitic diseases. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **70**, 98–106.

VOLLER, A., BIDWELL, D. E., HULDT, G. & ENGVALL, E. (1974). A microplate method of ELISA and its application to malaria. *Bulletin of the World Health Organization* **51**, 209–211.

WANG, S., ZENG, X. & YI, X. (1995). Purification of 31/32 kDa proteins of *Schistosoma japonicum* as antigens (Sj31/32) for ELISA and IHA. *Chung-Kuo-Chi-Sheng-Chung-Hsueh-Yu-Chi-Sheng-Chung-Ping-Tsa-Chih* **13**, 25–31.

WARREN, K. S. (1973). The pathology of schistosome infections. *Helminthological Abstracts* **42**, 591–593.

XU, H., REKOSH, D. M., ANDREWS, W., HIASHI, G. I., NICHOLSON, L. & LOVERDE, P. (1991). Schistosomiasis mansoni tropomyosin: production and purification of the recombinant protein and its immunodiagnostic potential. *American Journal of Tropical Medicine and Hygiene* **45**, 121–131.

XUE, C. G., TAYLOR, M. G., BICKLE, Q. D., SAVIOLI, L. & RENGANATHAN, E. A. (1993). Diagnosis of *Schistosoma haematobium* infection: evaluation of ELISA using a keyhole limpet haemocyanin or soluble egg antigen in comparison with detection of eggs of haematuria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 654–658.

YACOUB, A. A. H. & LILLYWHITE, J. E. (1985). The effect of chemotherapy on the serological response of patients with schistosomiasis haematobium infection using the enzyme-linked immunosorbent assay. *Journal of the Faculty of Medicine Baghdad* **27**, 19–29.

YE, X. P., DONNELLY, C. A., ANDERSON, R. M., FU, Y. L. & AGNEW, A. M. (1998). The distribution of *Schistosoma japonicum* eggs in faeces and the effect of stirring faecal specimens. *Annals of Tropical Medicine and Parasitology* **92**, 181–185.

YUESHENG, L., RABELLO, A. L. T., SIMPSON, A. J. G. & KATZ, N. (1994). The serological differentiation of acute and chronic *Schistosoma japonicum* infection by ELISA using keyhole limpet haemocyanin as antigen. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 249–251.

ZHENG, X. F., WANG, S. P., LA, W. Z., XIANG, Y. D. & YI, X. Y. (1992). Studies on the KLH-ELISA and KLH-IHA for the diagnosis of *Schistosoma japonicum* infection. *Chinese Journal of Schistosomiasis Control* **4**, 57–58.