

Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies?

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A simple, cheap, sensitive and specific assay for routine diagnosis of schistosome infection is not yet available. This review considers some of the advantages and disadvantages of parasitological microscopy, circulating-antigen detection and antibody-detection methods. Immunoassays for the detection of antibodies reactive against schistosome egg antigens have the potential to be useful in both clinical and epidemiological settings.

Schistosomiasis continues to be a serious worldwide public health problem. In Africa, particularly south of the Sahara, the prevalence, intensity and incidence of schistosomiasis is showing no decrease and 85% of the 200 million people with the disease are estimated to be living in that continent [1].

Methods that allow infections to be correctly diagnosed are a prerequisite for effective disease control. This applies not only to those living in endemic areas, but also to tourists and other travellers to the region who return home infected [2].

Schistosomiasis can be diagnosed by a direct or an indirect method. The two main variants of direct methods are: (i) the detection of parasite eggs in excreta or in the tissues of infected individuals; and (ii) detection of parasite-derived material in the circulation or excreta. Methods that involve detection of schistosome-specific antibodies constitute the bulk of indirect methods. There are other methods such as questionnaires and detection of haematuria that also come under the heading 'indirect', but their specificity is questionable, and relying on the presence of haematuria is an insensitive screen in lightly infected individuals (e.g. those with < 50 eggs per 10 ml urine) living in or travelling from areas infested with schistosomes.

Diagnosis by microscopy

Detection of excreted ova is the traditional and still very widely used diagnostic method of schistosome infections, with the infecting species identifiable 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 of parasitological methods, but the Kato–Katz thick smear [3] is the most extensively used method of examining stool for *Schistosoma mansoni*

and *Schistosoma japonicum* eggs, and sedimentation or filtration of urine is used for *Schistosoma haematobium* eggs.

Parasitological diagnosis has the advantages of high specificity and of requiring relatively unsophisticated equipment and, in areas of high endemicity, personnel with only basic training. It could therefore be the lowest cost option when technical assistance is plentiful. However, for some time, it has been recognized that parasitological detection methods lack sensitivity [4] and that egg counts are inaccurate. (See Box 1 for mathematical definitions of sensitivity and specificity.)

In Kato–Katz tests, as little as 50 mg of material are examined, which gives a sensitivity cut-off of ~ 20 eggs per g of faeces (epg). The factors that contribute to inaccuracy include uneven distribution of eggs in solid excreta [5], large day-to-day variations in infected individuals [6] and

Box 1. Calculation of the sensitivity of a diagnostic test

In the present context of evaluating the performance of immuno-diagnostic tests for schistosome infections, microscopical examinations for eggs in excreta are often used to indicate if infection is present or absent – i.e. parasitology is the gold standard against which new tests are compared.

Table I shows the relationships between the results given by a diagnostic test, and the presence or absence of infection.

Table I. Relationships between the results given by a diagnostic test, and the presence or absence of infection^a

		Infection	
		Present	Absent
Test result	Positive	True positive	False positive
	Negative	False negative	True negative

^aTable is adapted from Ref. [29].

A test's sensitivity equals the number of people who give a positive result as a proportion of the total number who have an infection (Eqn I) (see Table I for explanation of the parameters).

$$= \frac{\text{No. of true positives}}{\text{No. of true positive + false negatives}} \quad [\text{Eqn I}]$$

Test specificity equals the number of people who give a negative test result as a proportion of the total number without infection (Eqn II).

$$= \frac{\text{No. of true negatives}}{\text{No. of true negatives + false positives}} \quad [\text{Eqn II}]$$

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the operators' technical competence [7]. Many lightly infected individuals (<100 epg) are thus probably missed. Sensitivity and accuracy can be improved by increasing the size and/or number of samples examined, enhanced staff training and by tissue biopsy, all of which of course increase costs.

Errors due to low sensitivity in diagnosis will be exacerbated when infection intensities are low such as in areas of low prevalence, in individuals with early- or late-stage infections and after introduction of control measures that are effective in reducing transmission. Most cases of imported schistosome infections are also likely to fall in this category.

Direct immunological tests

It was anticipated that the difficulties associated with parasitological diagnosis might be overcome by adoption of immunological methods that provided direct evidence that an infection was present. Immunoassays had shown early on that schistosome-derived antigens were present in the circulation and/or excreta of infected hosts [8] and these observations prompted considerable research on their potential for immunodiagnosis of schistosomiasis.

Different schistosome-derived antigens have been found in the circulation, but two in particular have been worked on most extensively [9]: (i) circulating anodic antigen (CAA); and (ii) circulating cathodic antigen (CCA), named according to their migratory behaviour in immunoelectrophoresis. Both are proteoglycans and their chemical structure has been partially characterized [10,11], although their role in the host–parasite relationship is not yet known.

Methods for detecting circulating antigens generally involve capture of the antigen in an antibody 'sandwich', the antibodies being monoclonal or polyclonal with specificity for repeated epitopes on the antigens. Various assay methods have been used, including indirect haemagglutination [12], time-resolved immunofluorometric assay [13], magnetic bead immunoassay [14] and reagent strips [15].

Because of the shared carbohydrate epitopes, schistosome-derived circulating antigens are not species specific [16] and therefore this methodology cannot distinguish between infections of different species. Otherwise, diagnostic specificity is high because little false-positivity is found outside schistosome-endemic areas and circulating antigen levels generally correlate with excreted egg counts [17].

With regard to *S. mansoni* infection, the sensitivity of CAA detection by antigen capture enzyme-linked immunosorbent assay (ELISA) was estimated to compare well with a single faecal egg count [18], with the lower level of antigen detectability in serum corresponding to an egg excretion count of 10 epg of faeces [19]. The sensitivity of circulating antigen detection thus tends to be no better than that of parasitology-based methods, and could possibly be worse in areas of low infection prevalence and intensity [20], conditions which also apply to infected individuals who originate from non-endemic areas (i.e. in travellers' medicine) [21].

Diagnosis by detection of circulating antigens has potential advantages when compared with specific antibody detection. For example, both CAA and CCA can be detected in urine [22], the collection of which is less

invasive than the collection of blood. CAA and CCA are probably present only when there is active schistosome infection and should enable the effects of treatment to be assessed more accurately [23,24]. These advantages are to some extent compromised by an apparent failure to improve on the sensitivity of parasitological methods, the necessity of an additional pre-treatment step in the procedures for assaying urine or serum samples for circulating antigens that is not needed for antibody detection [25], and a requirement for different filter material (from that required for antibody detection) for collection of dried blood samples [26].

Finally, in the context of detection of parasite-derived material, the potential for detecting schistosome DNA in faeces by a polymerase chain reaction (PCR) is being investigated [27].

Indirect immunological tests

A diagnostic test should be both sensitive and specific, each of which is defined mathematically [28]. Box 1 shows how the results of a test that is being developed relate to the occurrence of infection or disease, and how sensitivity and specificity are calculated.

There is generally a trade-off between sensitivity and specificity, and the relative performance of a new diagnostic test is therefore calculated as an index in which both these parameters are accounted for. However, as stated by Fletcher *et al.* [29]: 'Assessment of the test's accuracy rests on its relationship to some way of knowing whether a disease is truly present or not – a sounder indication of the truth often referred to as the "gold standard". As it turns out, the gold standard is often elusive.' This problem impinges acutely on diagnostic tests based on antibody detection because the gold standard has nearly always been parasitological microscopy and, as suggested above, these methods could be defective because of a lack of sensitivity. One paradoxical consequence of this is that antibody detection tests are often reported to have low specificity (i.e. they give false-positive results).

Patent schistosome infections are highly immunogenic and there is no difficulty in demonstrating the presence of anti-schistosome antibodies or cell-mediated immune responsiveness in infected subjects. Many different assays have been used to display such immunological reactivity, including skin hypersensitivity reactions against injected antigens, complement fixation, indirect immunofluorescence, indirect haemagglutination, radioimmunoassay, and various flocculation and precipitation tests (for reviews, see Refs [30–33]). Currently, ELISA are used extensively.

ELISA was first described by Engvall and Perlmann [34] in 1971, since when many different variations of the basic theme have been developed for diagnosis, although its adaptation for use in microtitration plates as described by Voller *et al.* [35] remains the most popular. Antigens from virtually all stages of the schistosome life cycle have been tested for immunodiagnostic potential. Some tests use more-or-less intact forms of the parasite (e.g. the cercaricercarial reaction, the circumoval precipitin test and indirect immunofluorescence on adult worm sections), and extracts from these stages and fractionated derivatives thereof have also been tested in ELISA.

The antigens of schistosome larvae have not found favour for diagnostic purposes because of inferior sensitivity and specificity when compared with those from worm or egg antigens. A higher ratio of anti-larval to anti-adult antibody could, however, be useful in discriminating between acute and chronic schistosome infections [36].

Adult worms have the advantage of being the most abundant and easily obtained source of antigenic material. Crude extracts of worms work well in ELISA [37], and worm antigens generally give higher sensitivity and specificity than those from larvae [38]. Purified and recombinant antigens have been proposed to perform better than crude extracts in ELISA, and/or purified antigens could provide additional information relating, for example, to age of infection, distinctive pathology or protective immunity. Distinct worm preparations that have been considered diagnostically useful include a microsomal extract [39], gut-associated polysaccharide [40], heat-shock protein 70 [41], and worm antigens Sm31 and Sm32 [42]. A variation of the enzyme immunoassay exploits the antigenicity and enzymatic activity of schistosome worm alkaline phosphatase which is captured by infected patients' antibody bound via protein A to a solid matrix [43].

In experimental hosts, anti-schistosome antibody reactivity remains low, even against larval and worm antigens, until the infections become patent [44,45]. This could be a result of the early stages of infection being poorly immunogenic, which, in turn, helps explain why a good test to diagnose pre-patent infections has not yet been devised. By contrast, schistosome eggs are highly immunogenic – their exit from the host after all depends on it [46] and, in consequence, anti-schistosome antibody titres rise after the onset of infection patency, as defined by the detection of eggs in clinical specimens. The fact that after patency there is an increase in anti-larval and anti-worm, in addition to anti-egg, antibody titres is perhaps best explained by the production (at least initially) of antibodies specific for glycanic epitopes which schistosome larvae and worms, and probably also other parasites [47], have in common. Keyhole limpet haemocyanin (KLH) also possesses carbohydrate epitopes that are cross-reactive with those of schistosomes [48,49], which explains why KLH has been proposed as a diagnostic reagent for schistosome infection [33].

Results from individual laboratories and, more importantly from multicentre trials, suggest that egg antigens provide greater diagnostic sensitivity and specificity than worm antigens for the detection of an infection [38,50,51]. ELISA with non-fractionated extracts of *S. mansoni* eggs has been recommended or is now routinely included in diagnostic tests for schistosome infections in travellers [2,52].

Extracts prepared by homogenizing schistosome eggs [conventionally known as soluble egg antigens (SEA)] contain a large number of molecules, although only a minority of the constituents of SEA might be released by viable eggs *in vivo*, as demonstrated *in vitro* [53]. Consistent with this notion, antibodies in humans infected with *S. mansoni* are particularly reactive against only a limited number of antigens in SEA, whereas specificity is indicated by sera obtained from patients with other parasitic infections showing little reactivity (see Figure 2

in Ref. [33]). The pattern of reactivity of sera from *S. mansoni*-infected patients is consistent with the rationale of using purified antigen preparations for diagnosis by antibody detection because *S. mansoni* infection sera react mainly against three antigens identified as: (i) 30 kDa ω -1; (ii) a 36–41 kDa antigenic doublet named α -1; and (iii) >90 kDa κ -5. The antigens α -1 and ω -1 constitute a fraction, CEF6, which can be purified by a one-step cation-exchange chromatography method [45] and, in a multicentre trial, CEF6 produced sensitivity and specificity values of 91% and 90%, respectively [50].

Progress in exploitation of the potential of recombinant DNA technology to produce antigens useful for schistosomiasis diagnosis has been slow [33]. Most recently, a microbial expression system has been used to produce a recombinant form of an interleukin-4 (IL-4)-inducing factor from *S. mansoni* eggs (IPSE), which induces basophils to degranulate during *in vitro* culture [54]. IPSE has been found to be the same as antigen α -1 of CEF6 (G. Schramm, H. Haas and M. Doenhoff, unpublished) and the serodiagnostic potential of IPSE/ α -1 is currently being evaluated. There are, however, difficulties regarding the use of microbially expressed recombinant antigens for serodiagnosis of schistosomiasis: (i) such antigens will not be glycosylated or necessarily folded correctly, but much of the antibody produced initially in response to schistosome infection reacts against carbohydrate epitopes; and (ii) specificity could be compromised if the recombinant antigens cannot be fully separated from other constituents of microbial cells to which infected subjects might also have produced antibodies.

Potential solutions to these problems exist, for example, use of eukaryotic cell lines or enzymatic synthesis to produce glycoconjugates, novel buffer combinations to enable correct protein folding, and expression of His-tagged proteins to facilitate purification.

Advantages and disadvantages

Lack of sensitivity is a problem common to both parasitological and antigen-detection methods of diagnosis. Solutions are available, for example, differential concentration of eggs from faecal matter [55] or mathematical modelling [56], respectively, but these could add cost in terms of time and/or material resources. The medical need to detect low-level intensity infections could in any case be questioned because they are generally not associated with severe disease symptoms and do not therefore require urgent treatment. Sensitivity could, however, have more relevance in a public health context; for example, in situations where control has resulted in very low levels of infection intensity and prevalence, and the goal is complete elimination.

The most common criticisms of antibody-detection methods are: (i) they do not accurately reflect the presence of active infection and results are therefore potentially flawed when compared with direct methods such as parasitology and antigen detection. This is considered to be a particular problem in the context of monitoring the effects of chemotherapy. (ii) They do not reflect infection intensity. (iii) They have poor specificity (i.e. a high proportion of egg-negative, antibody-positive results), particularly in endemic areas.

Excreted egg counts and circulating-antigen

concentrations do indeed decline rapidly after chemotherapy [57], whereas antibody levels can remain high [2,58]. However, this problem could be alleviated by the use of purified antigens such as CEF6, antibodies against which decline more rapidly than those against SEA. Thus, in an interlaboratory trial of different antigen preparations, CEF6 was the only antigen preparation to show a significant decline in antibody titres after chemotherapy [50] and in a study in Kenya the reduction in anti-CEF6 antibody levels six months after drug treatment was significantly greater than that of anti-SEA antibodies [59]. Therefore, the apparent problem of post-treatment persistence of antibody could be alleviated by the use of a selected antigen preparation such as CEF6, together with assays for antibody isotypes that show more rapid decrease after chemotherapy, such as immunoglobulin (Ig) A [58] or IgG4 [60].

A possible reason for antibody levels remaining high after chemotherapy is that the drug dosage routinely used to treat infected subjects might not always eliminate infection completely [61]. Many treatments could therefore be sufficiently effective to cause excreted egg counts and circulating-antigen concentrations to decrease below easily detectable levels, but still leave a residue of infection that is sufficient to maintain antibody positivity. Nevertheless, following treatment, antibody titres do decrease commensurately with reductions in infection intensity [59]: assay for antibody activity before and on a regular basis after implementation of a chemotherapy-based control programme could therefore provide useful information on the programme's effectiveness.

Better correlations with infection intensity could be obtained through the use of purified antigens such as CEF6 [50] and/or assaying for particular immunoglobulin isotypes [60]. On an epidemiological level, the graphical patterns for age–intensity and age–prevalence relationships given by serological results can reflect parasitological data closely [62]. The perceived failure of serological results to correlate exactly with infection intensities could in any case be due in part to the inherent inaccuracies of parasitological techniques.

Similarly, the high proportion of antibody-positive, but egg-negative individuals which give the ostensibly poor specificity that is often found in samples from endemic areas and that is also found in travellers [2] could be largely attributable to the lack of sensitivity of the gold standard, microscopy.

Concluding remarks

A substantive Schistosomiasis Control Initiative (SCI) funded by the Bill and Melinda Gates Foundation (<http://www.schisto.org/mission.htm>) is being implemented in selected sub-Saharan African countries to target chemotherapy to people at severe risk from disease morbidity. This will probably provide a good opportunity to investigate whether serological tests can be used to monitor progress during efforts to control schistosomiasis.

The trials organized by WHO and published by Mott and Dixon in the 1980s [50,63] were an early acknowledgement that methods of diagnosing schistosomiasis needed to be improved and standardized. Their objectives have not yet been fulfilled, and the need has been made more acute by the

past twenty years of morbidity reduction in many parts of the world [64,65]. It is reassuring that WHO is now generating interest in a new round of trials finally to accomplish the task (R. Bergquist, pers. commun.).

With regard to areas of low infection prevalence and intensity in particular, conventional parasitological methods for monitoring infection might not be effective because of their low sensitivity, labour intensiveness and a loss of motivation in personnel having to examine samples that are predominantly negative. Would specific anti-schistosome antibody detection be a suitable replacement? We believe so because, according to the current state-of-the-art, people who are parasitologically negative and/or circulating antigen-negative cannot be assumed to be non-infected. By contrast, populations that have no anti-schistosome antibodies will be free of infection.

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