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Use of Schistosoma mansoni soluble egg antigen (SEA) for antibody detection and diagnosis of schistosomiasis: The need for improved accuracy evaluations of diagnostic tools

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ABSTRACT

Many antigens for use in antibody-detection systems for schistosomiasis have been investigated over the past 40 years. In particular, soluble egg antigens (SEA) are still widely used in enzyme-linked immunosorbent assays (ELISAs) for detection of immunoglobulin classes and subclasses. Here, we conducted a literature review to examine accuracy evaluations of SEA-Immunoglobulin G (IgG)-ELISAs performed to detect *Schistosoma mansoni* infections and published between 1979 and 2019. *S. mansoni* is the main causative agent for intestinal schistosomiasis in many countries in Africa and Central and South America. After retrieving 214 relevant abstracts from the PubMed database, we selected 15 publications to undergo a full review. Sensitivity and specificity values varied from 71 to 99%, and from 6 to 100%, respectively. In addition, 11/15 studies did not state confidence intervals. Therefore, the findings from this review indicate that after four decades, we still do not have consistent evaluation estimates of SEA-IgG-ELISAs. Antigen mass per well and dilution of test sera in these articles varied from 0.018 μg to 1.5 μg, and from 1:50 to 1:500, respectively. Most of the reported accuracy evaluations used control sera which were selected based on parasitological examinations for egg detection, although ill-defined criteria were also noted. The number and composition of control serum panels was considered not adequate in approximately half of the studies. It is also noteworthy that among more than 30 diagnostic antigen preparations under development since the 1970s, most were not validated in the field and they failed to reach populations in need. Thus, attention to guidelines for standardization, estimations of accuracy, and reporting of results is needed to facilitate coordinated efforts aimed at schistosomiasis control and elimination.

1. Introduction

There has been a long standing and coordinated effort by the World Health Organization (WHO) to effectively control and eliminate human schistosomiasis (WHO 1993, 2013). As a result, the prevalence and intensity of schistosomiasis infections in many localities worldwide have decreased (Stothard et al., 2014; WHO, 2013; Lo et al., 2017). However, elimination of schistosomiasis remains an ongoing goal (WHO, 2013). It has also been established that sensitive diagnostic tools are required to eliminate transmission of schistosomiasis (McLaren et al., 1979; Mott and Dixon, 1982; Stothard et al., 2014). With an increasing number of travelers between endemic and non-endemic countries, there has been a

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growing concern that individuals who are lightly infected and remain negative by routine egg detection methods require more stringent screening (Corachan, 2002).

The Kato-Katz method (KK) is a parasitological method which is recommended for screening, identifying, and treating intestinal schistosomiasis (WHO, 1985). However, its lack of sensitivity in diagnosing low intensity infections is a major drawback (Tosswill and Ridley, 1986; de Vlas and Gryseels, 1992; van Gool et al., 2002; Cavalcanti et al., 2013; Stothard et al., 2014). This limitation compromises its ability to monitor areas and achieve successful control and interruption of transmission. More recently, rapid diagnostic tests for antigen detection in urine have emerged as a substitute for parasitological examinations and as useful tool for epidemiological monitoring (WHO, 1993; Shane et al., 2011; Colley et al., 2020).

Considering the difficulties associated with parasitological examinations, serological methods have been extensively studied over the past four decades as possible alternatives (Doenhoff et al., 1993, 2004; Weerakoon et al., 2015; Hinz et al., 2017). The development, evaluation and application of serological methods have been recommended by the WHO, concomitant with a progressive shift from morbidity control toward transmission interruption and post-elimination vigilance (WHO 1993, 2009a, 2013).

Over a two-year period (1980–1981), a collaborative multicenter study was coordinated by the WHO to evaluate 10 antigens and 8 techniques for anti-*Schistosoma* antibody detection (Mott and Dixon, 1982). This initiative was a unique milestone which has never been repeated, nor extended, over many decades of searching for new diagnostic targets. This study included well-defined protocols for antigen preparation, and test sera examinations were performed in ten participating laboratories in five countries (United States ($n = 5$), England ($n = 2$), France, Netherlands, and Sweden). Each participating laboratory received the same set of serum aliquots, with sera obtained from infected individuals in Brazil, Kenya, and the Philippines. Proper standardization and evaluation of diagnostic test performance requires collaborative arrangements, quality control and good laboratory practices (GLP), similar to those reported by Mott and Dixon (1982). This multicenter study also implicitly stresses serology as a useful diagnostic tool for schistosomiasis.

Among the many antigenic preparations generated, crude soluble egg antigen (SEA) has been used worldwide, mainly for IgG detection in enzyme-linked immunosorbent assays (IgG-SEA-ELISA) (Mott and Dixon, 1982; Doenhoff et al., 1993, 2004; Ferrer et al., 2020). Here, we conducted a literature review to examine accuracy evaluations of SEA-IgG-ELISAs performed to detect *Schistosoma mansoni* infections between 1979 and 2019. We selected 15 out of 214 reports which had the objective of verifying compliance of IgG-SEA-ELISAs with fundamental standard operational procedures for development, accuracy evaluation and reporting. We focused on diagnosis of *S. mansoni* since it is the main causative agent for intestinal schistosomiasis in many countries of Africa and Central and South America (WHO, 2013; Zoni et al., 2016).

2. Serology for diagnosis of schistosomiasis

The advantages of antibody detection systems include high sensitivity, relatively straightforward automation, and large scale processing of samples (Doenhoff et al., 2004; Weerakoon et al., 2015). However, these systems also have several limitations: i) antibodies may circulate for a long time after an infection is cured (Capron et al., 1969; Tosswill and Ridley, 1986); ii) cross-reactivity between antigens from *Schistosoma* species and other helminths may occur (Alarcón-de-Noya et al., 1996); iii) difficulties with large scale production may be encountered; iv) some techniques have significant costs and require complex equipment; v) there are operational complexities associated with blood collection and the transportation and storage of sera; and vi) there may be difficulties in evaluating performance (Doenhoff et al., 2004; Hamilton et al., 1998). Lack of reproducibility between different batches of reagents may further hinder or delay implementation of new diagnostics (Banoo et al., 2006). These challenges have been observed with the development of the point-of-care circulating cathodic antigen (POC—CCA) detection test for urine (Silva-Moraes et al., 2019; Viana et al., 2019).

Over the past four decades, approximately 32 *S. mansoni* antigenic preparations have been evaluated as probes to detect antibodies in various systems. In particular, these preparations have been used in ELISAs and indirect hemagglutination assays (IHA) (Mott and Dixon, 1982; Hinz et al., 2017). Since the late 1970s and early 1980s, crude SEA or fractions of SEA were preferentially used as diagnostic antigens (McLaren et al., 1978, 1979; Mott and Dixon, 1982; Ferrer et al., 2020).

3. Lack of a gold standard

The assumption that a "gold standard" is equivalent to a "reference method" is controversial. A key reason why these are not considered equivalent is that a test with 100% sensitivity and specificity, a "fail-safe method", or "gold standard", may not exist (Cavalcanti et al., 2013; Stothard et al., 2014; IOS, 2007). However, there are reasons to accept the definition of "gold-standard" as the method, or set of methods, which currently present the best performance (Irwig et al., 2002; Banoo et al., 2006). Correspondingly, the "International vocabulary of basic and general terms in metrology" (ISO VIM) defines a standard reference method as: the method capable of identifying a number of positive samples, the closest possible to the true number of true-positive samples (IOS, 2007).

Lack of a direct gold standard for evaluating comparative performance of new immunological or molecular methods has been a central problem for decades. Demonstration of eggs in stools is considered a definitive and confirmatory diagnosis for schistosomiasis (Mott and Dixon 1982; Corachan 2002; Kinkel et al., 2012). However, parasitological methods have limitations. For example, false-negativity may result from sparseness of eggs in large volumes of excreta and from uneven egg distribution and their intermittent release in feces (Engels et al., 1997). Nevertheless, it is important to stress that the KK method is a simple and cost effective procedure. It has been recommended by the WHO for screening populations, especially at the stage of morbidity control (WHO, 1985). However, the KK method does not represent a reference or "gold" standard, as some researchers incorrectly state (Sarhan et al., 2014; Silveira et al., 2016). Moreover, the descriptive term "gold standard" sometimes is loosely and incorrectly applied to a method that is "widely used" or "recommended" (Cavalcanti et al., 2013).

Methods for detecting molecular markers, such as antibodies, are expected to exhibit greater sensitivity. However, appropriate evaluations of these methods are hindered by the absence of a highly sensitive confirmatory parasitological method. As a result, classification of "false-negatives" may occur due to an insufficiently sensitive gold standard (Mott and Dixon, 1982). Doenhoff and collaborators (1993) presented detailed data to demonstrate the trade-off between specificity and sensitivity (see Fig. 6 in their publication). They show that highly sensitive molecule detection methods may present low specificity, while modifications to raise specificity may compromise sensitivity, and vice-versa.

When there are no direct confirmatory methods with acceptable sensitivity and specificity, or an insufficiently accurate reference method, two alternatives may be considered: i) use of a "consolidated reference standard" (CRS) and ii) a latent class analysis (LCA). A CRS is established when the identification of an infection from several methods are taken together to establish a "gold-standard" (Banoo et al., 2006; Oliveira et al., 2018). Meanwhile, LCA is a stochastic modeling approach which estimates the number of true-positive individuals in a population. The latter is based on prevalence resulting from the application of diagnostic tests under evaluation (Pepe 2003; Smeden 2014).

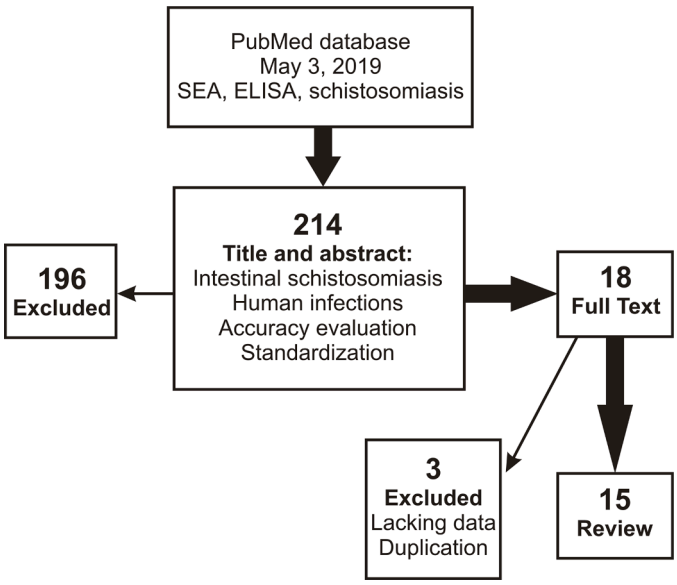

Fig. 1. Flow diagram of literature PubMed search with keywords "SEA", "ELISA" and "schistosomiasis" in May 3, 2019 in order to review standardization and accuracy evaluations of SEA-ELISA for diagnosis of intestinal schistosomiasis. Two selection steps followed and resulted in full review of 15 papers.

Helmintex (HTX) is a new and highly sensitive reference method (Teixeira et al., 2007; Favero et al., 2017). It is based on the ability to concentrate and isolate *Schistosoma* eggs in the presence of paramagnetic beads. As a result, *Schistosoma* eggs can be isolated from large volumes of feces. In seeding experiments, 100% sensitivity was achieved for egg numbers greater than 1.3 eggs per gram. HTX is currently the best method for identifying true-positive samples when evaluating other methods (Lindholz et al., 2018; Oliveira et al., 2018). It is noteworthy that the use of such a highly sensitive egg-detection method resulted in LCA estimates of performance which did not significantly differ from those produced by the egg-detection method itself (Lindholz et al., 2018).

4. *S. mansoni* SEA

Since the late 1970s, SEA has been recognized as a preparation which is useful for a diagnosis of schistosomiasis (McLaren et al., 1978, 1979). Eggs isolated from livers of experimentally infected mice are homogenized and submitted to centrifugation. The resulting clear supernatant is named SEA (Boros and Warren, 1970). This antigen preparation is still widely used by many clinical and research laboratories which employ both in-house and commercial ELISA kits, and is used almost exclusively for detection of IgG antibodies (Kinkel et al., 2012; Hinz et al., 2017). Crude SEA, or its fractions, have been employed in other antibody detection systems as well, including IHAs, radioimmunoassays (RIAs), indium slide immunoassays (ISIs), and the circumoval precipitin test (COPT) (Mott and Dixon, 1982; Hinz et al., 2017). However, these systems are not routinely used, except in a few studies and laboratories which perform IHAs and COPTs (Kinkel et al., 2012; Espírito-Santo et al., 2014).

5. Literature review of SEA-IgG-ELISA

A search was conducted of the PubMed database on May 3, 2019 which included the following set of keywords: "Schistosomiasis", "SEA", and "ELISA". A total of 214 publications were retrieved from 1979 to 2019 (Fig. 1). Based on titles and abstracts, 196 publications did not address evaluations of SEA-ELISAs and these were excluded. A full reading of the remaining 18 reports led to the exclusion of an additional three papers due to a lack of sufficient data or because they were short communications followed by a full report (Beck et al., 2004, 2008). An extensive review of the remaining 15 publications was guided by a protocol for collecting relevant information (Tables 1–6).

Table 1
Antigens under evaluation other than soluble egg antigen (SEA) for diagnosis of schistosomiasis, in 15 studies conducted between 1979 and 2019.

Reference	Antigens under evaluation besides SEA
McLaren et al., 1979	none
Mott and Dixon, 1982	several egg and adult worm antigens
Tosswill and Ridley, 1986	none
Doenhoff et al., 1993	CEF6
Alarcón-de-Noya et al., 1997	APIA
van Gool et al., 2002	AWA
Sorgho et al., 2005	CEF6, SWAP
Beck et al., 2008	SWAP, KLH
El-Aswad et al., 2011	SmCTF, rSmCRT
Stothard et al., 2011	none
Kinkel et al., 2012	SCA, AWA
Smith et al., 2012	SmCTF
Grenfell et al., 2013	SWAP
Dawson et al., 2013	SmCTF
Sarhan et al., 2014	SWAP, SCA

CEF6: cationic exchange fraction 6 from *S.mansoni* eggs; APIA: Alkaline Phosphatase Immunoassay; AWA: adult worm antigen; SWAP: soluble *S.mansoni* worm antigen; KLH: keyhole limpet hole antigen; SmCTF: *S.mansoni* cercaria transformation fluid; r-SmCRT: recombinant *S.mansoni* calreticulin; SCA: soluble cercarial antigen.

Performance of SEA-ELISAs was not always the main objective of the studies selected. In some of the publications, the test was used as a reference method for evaluating other tests or specific applications (Smith et al., 2012; Alarcón-de-Noya et al., 1997; Dawson et al., 2013). For example, SEA-ELISAs were used to evaluate the discriminative ability of tests for acute versus chronic infections (Beck et al., 2008) (Table 1).

6. Antigen preparations

It has been observed that SEAs obtained from different *S. mansoni* strains do not produce a significant difference in SEA-ELISA performance (Doenhoff et al., 1993). It is possible that multiple variables in the preparation of antigen contribute to this result, and we will not address this further here. In a collaborative multicenter study sponsored by the WHO, all participating laboratories received the same antigens and serum samples for testing with well-established standard operational procedures (Mott and Dixon, 1982).

Among the 15 studies we reviewed, antigen mass per well and dilution of test sera were found to vary widely (e.g., 0.018 μg to 1.5 μg for antigen mass, and 1:50 to 1:500 for dilution of test sera) (Table 2). These are critical characteristics for any antibody detection system, and their variability prevents any consistent appraisal of test performance. An additional problem is the lack of other relevant data in the product information provided by the commercial sources of the SEA-ELISA kits used. In particular, information regarding antigen source and preparation, as well as protein mass per well, are incomplete (Kinkel et al., 2012; Dawson et al., 2013).

The lack of compliance with guidelines for standardized reporting of SEA-ELISA evaluations also prevents the analysis of several other variables that may affect the accuracy of a study. For example, the method for serum sample collection and storage, the characteristics of different ELISA plates, and controls to guarantee inter-ELISA-plate reproducibility can affect the accuracy of ELISA results (Tosswill and Ridley, 1986; Doenhoff et al., 1993; van Gool et al., 2002).

Table 2
Type of ELISA kits (commercial or in-house), antigenic mass per well, dilution of test sera, and estimates of sensitivity and specificity of SEA-IgG-ELISAs. Data are from 15 studies published between 1979 and 2014.

Reference	Commercial Kit or inhouse ELISA	Antigen mass (μg) per well	Dilution of test sera	Sensitivity (%) and Confidence Interval	Specificity (%) and Confidence Interval
McLaren et al., 1979	In house	0.25	1:300	99	100
Mott and Dixon, 1982	in house	0.1	1:500	84	94
	In house	0.2	1:500	79	68
Tosswill and Ridley, 1986	In house	0.25	1:300	96	97
Doenhoff et al., 1993	In house	1.5	1:150	94	64
Alarcón-de-Noya et al., 1997	In house	0.5	1:200	98	37
van Gool et al., 2002	In house	0.039	1:400	93	98
Sorgho et al., 2005	In house	0.1	1:100	96	26
Beck et al., 2008	In house	1.0	1:200	84 (76–90)	64 (35–87)
El-Aswad et al., 2011	In house	0.1	no information	90	57
Stothard et al., 2011	IVD Carlsbad	no information	1:40	93 (83–98)	69 (62–76)
Kinkel et al., 2012	Inhouse	0.018	1:100	90 (68–98)	97 (91–99)
	Commercial, DRG	No information	1:100	86 (62–96)	88 (80–93)
	Commercial, Viramed	No information	1:100	71 (47–87)	76 (67–84)
Smith et al., 2012	Commercial BioGlab	0.025	1:300	92	Not done
Dawson et al., 2013	Commercial ScidMedx	No information	1:40	77 (46–95)*	38 (20–57)*
	Commercial ScidMedx	No information	1:40	87 (67–97)**	6 (0–30)**
Grenfell et al., 2013	Inhouse	0.3	1:150	85	80
Sarhan et al., 2014	Inhouse	0.5	1:100	80	76
	Inhouse	0.5	1:50	80	82

* Children under 3 years-old.
** children of 4 and 5 years-old.

7. Sizes of serum panels and definitions of true-positive versus true-negative samples

Samples were collected from infected populations in endemic (11/15) and non-endemic areas (3/15) (Table 3). In one study, both endemic and non-endemic populations were sampled (Table 3). Similarly, negative control serum samples were obtained from non-endemic (5/15), endemic (4/15), and both endemic and non-endemic areas (4/15). However, in one study, the site of sample collection was not provided, while a negative control panel was absent in another study (Tables 2 and 4). In addition, the origins of the individuals donating serum samples from the endemic and non-endemic territories were not provided. This is an important source of misclassification, especially for the negative control sera, because there is a higher prevalence of other parasitic infections in individuals from endemic countries (El-Aswad et al., 2011). Furthermore, long lasting persistence of antibodies has been observed following a cure of schistosomiasis (Capron et al., 1969; Tosswill and Ridley, 1986).

The number of positive control sera varied from 13 to 618 among the studies examined. The distribution of these sera is represented in Fig. 2 and Table 5. True-positive samples were defined based on the results of highly specific methods, including demonstration of eggs in stool for intestinal schistosomiasis (Mott and Dixon, 1982). True-negative samples are also best defined by a parasitological examination (Table 4). All but one study used egg detection in stool as the sole criteria to classify serum samples as true-positive (Table 5). Meanwhile, Sarhan and collaborators (2014) relied on both clinical and epidemiological information as criteria. Eggs were detected mainly by the KK method, with 2, 4, or 18 slides. In three studies, the parasitological method used was not disclosed (Table 5). The sensitivity limitations of classical parasitological methods affect the definition of true negative samples and prevent adequate accuracy estimation for molecular methods. Helmintex and the Saline Gradient methods, as highly sensitive egg detection methods, may contribute to overcome those limitations (Coelho et al., 2009; Oliveira et al., 2018).

The number of negative control sera varied from 15 to 1438, with the distribution shown in Fig. 2 and Table 2. An absence of *S. mansoni* eggs in stool was the best criterion. In six studies, this was the only criterion, while in six other studies, an absence of *S. mansoni* eggs in stools in combination with clinical-epidemiological criteria were the criteria used. In the remaining three studies, only ill-defined clinical or epidemiological criteria were employed, with identification of "healthy" individuals (Table 4).

The sample size of 97 for each category achieves the recommended sample size to allow comparisons of two proportions with 80% power and alpha 0.05 (95% confidence). Approximately half of the studies had inappropriate numbers of positive (55%) and negative (44%) control serum sets (Fig. 2).

Without a sufficient number of serum samples, a good estimation of performance parameters cannot be achieved. Briefly, sensitivity estimation is performed by comparing proportions of samples with a positive result by ELISA divided by the total number of true-positive samples (Banoo et al., 2006). Sample size is also important for representing the spectrum of reactivity, i.e., from very low to highly reactive. It is more important to have a serum panel from individuals with a wide range of egg numbers than sample panels which are "representative of endemic populations". Mott & Dixon (1982) called attention to a possible bias in their study, in which a majority of serum samples included in their panel were from highly infected and more severely diseased individuals. While searching for new antigens, it is recommended to avoid testing only "highly reactive sera", which is sometimes obtained from the experimental infection of animals (Dunne et al., 1984). It is possible that the latter is the reason why efforts to obtain a good diagnostic antigen for use in humans have been unsuccessful.

Negative control sera data are essential for evaluating specificity, defined as the number of negative test results as a proportion, or percentage, of the true-negative individuals as determined by the reference method (for several important and basic concepts definitions, see Banoo et al., 2006). Two different sources for negative control sera are: i) "healthy" or "normal negative", presenting very low reactivity; and ii) individuals ("healthy" or not) infected with other parasites, "specificity controls", as source of unspecific reactivity. "Healthy", as well as "normal", are problematic definitions, especially in endemic settings. In both categories, there are two subsets: a) individuals from endemic areas and b) individuals from non-endemic areas. Few studies have paid attention to these important details for adequate characterization and composition of reference panels. Data on control sera from individuals infected with other parasites are presented in Table 6. We do not make any comments regarding the latter due to variability in the methods used to characterize these samples. Mott and Dixon (1982) commented on

Table 3
Serological diagnosis of *Schistosoma mansoni* infections. Geographic origin and epidemiological settings of positive and negative control serum samples used in SEA-IgG-ELISAs performed in 15 studies published between 1979 and 2014.

Reference	Origin of positive control sera		Origin of negative control sera	
	Endemic (E) or Non-Endemic (NE) area	Location	Endemic or Non-Endemic area	Location
McLaren et al., 1979	Endemic	Saint Lucia Island, Central America	Non-endemic	Saint Vincent Island, Central America
Mott and Dixon, 1982	Endemic	Brazil, Phillipines and Kenya	Endemic and Non-Endemic	Endemic: undisclosed geographical origin of 10 KK negative samples
Tosswill and Ridley, 1986	Endemic and Non-endemic	UK, Hospital for Tropical Diseases, London: travellers	Endemic and Non-Endemic	UK, Hospital for Tropical Diseases, London: travellers
Doenhoff et al., 1993	Endemic	Kenya: Kamanzi (Machakos) and Taita/ Taveta	Non-Endemic	Kenya: Kericho: area without transmission
Alarcón-de-Noya et al., 1996	Endemic	Venezuela, Carabobo, Los Naranjos	Non-endemic	Venezuela, Bolívar State, Aripao: area without transmission
van Gool et al., 2002	Non-endemic	Netherlands and Belgium, clinics and hospitals	Non-Endemic	Netherlands and Belgium, clinics and hospitals
Sorgho et al., 2005	Endemic	Burkina Faso, Kou River Valley	Endemic	Burkina Faso, Kou River Valley
Beck et al.,	Endemic	Brazil: Pernambuco, Porto de Galinhas and São Lourenço da Mata	Undisclosed precise location	Probably also from Pernambuco, Brazil
El-Aswad et al., 2011	Endemic	Egypt, Northern Nile Delta	Endemic and Non Endemic	Egypt, Northern Nile Delta and
Stothard et al., 2011	Endemic	Uganda, Lake Victoria, Bugoigo	Endemic	Uganda, Lake Victoria, Bugoigo
Kinkel et al., 2012	Non-endemic	Germany, Institute of Tropical Medicine and International Health, Berlin	Non-endemic	Germany, Institute of Tropical Medicine and International Health, Berlin
Smith et al., 2012	Non-Endemic	United Kindom, London School of Tropical Medicine: travelers to African countries	No negative control sera was studied	No negative control sera was studied
Grenfell et al., 2013	Endemic	Brazil, Pedra Preta, Montes Claros	Endemic and Non-Endemic (*)	Brazil, Pedra Preta, Montes Claros (endemic)
Dawson et al., 2013	Endemic	Uganda, Lake Albert, Buliisa,	Endemic	Uganda, Lake Albert, Buliisa,
Sarhan et al., 2014	Endemic	Walukuba and Piida Egypt, Ain Shams University Hospital, Cairo	Endemic	Walukuba and Piida Egypt, Ain Shams University Hospital, Cairo

Table 4
Serological diagnosis of *Schistosoma mansoni* infections. Sample size and criteria for classification of true-negative serum samples in 15 studies conducted to evaluate SEA-IgG-ELISA performance between 1979 and 2019.

Reference	Sample size	Criteria for true negative control sera	Detailed criteria
McLaren et al., 1979	100	Epidemiological Absence of eggs in feces	Non-endemic Bell filtration method
Mott and Dixon, 1982	31	Epidemiological and Absence of eggs in feces	Non-endemic: Amazon (no transmission) and "Europeans" Endemic: Kato Katz, 20–40 slides and no skin reactivity (intradermal hypersensitivity reaction)
Tosswill and Ridley, 1986	1438	Epidemiology Absence of eggs in feces	Non-endemic, no travel Ritchie, 3 stools
Doenhoff et al., 1993	254	Absence of eggs in feces	Kato-Katz, 2 slides
Alarcón-de-Noya et al., 1997	116	Absence of eggs in feces	Sedimentation
van Gool et al., 2002	283	Epidemiology Absence of eggs in feces	"Healthy", non-endemic area Unspecified parasitological method
Sorgho et al., 2005	215	Absence of eggs in feces	Kato-Katz, 2 slides from 2 stools
Beck et al., 2008	15	Clinical, epidemiological Absence of eggs in feces	"Healthy" Kato-Katz and Hoffman-Pons-Janer (HPJ) (3 stools)
El-Aswad et al., 2011	92	Epidemiology and Absence of eggs in feces	Unspecified parasitological method
Stothard et al., 2011	213	Absence of eggs in feces	Kato-Katz 2 slides + FLOTAC + Percoll
Kinkel et al., 2012	104	Epidemiological	Non-endemic, no travel history
Smith et al., 2012	0**	Epidemiology	"No history of exposure"
Grenfell et al., 2013	20	Absence of eggs in feces	Kato-Katz, 18 slides
Dawson et al., 2013	27 and 16*	Absence of eggs in feces	Kato-Katz, 4 slides
Sarhan et al., 2014	16	Clinical	"Healthy"

* the main objective was to compare reactivity as measured by SmCTF-ELISA and SEA-ELISA and authors avoid to estimate specificity;.
** 27: children less than 3 years-old and 16: 4 and 5 years-old children.

their extensive characterization of samples, including information about other helminthic infections. However, these data are not included in their results and analysis. Evaluation studies should include adequate numbers of both "normal negative" and "specificity control" serum samples. Collecting a well characterized and properly handled and

Table 5
Serological diagnosis of *Schistosoma mansoni* infections. Sample size and criteria for classification of true-positive serum samples in 15 studies conducted to evaluate SEA-IgG-ELISA performance between 1979 and 2019.

Reference	Sample Size	Criteria for true positive control sera	Parasitological Methods
McLaren et al., 1979	213	Egg in feces	Bell filtration
Mott and Dixon, 1982	395	Egg in feces	Kato-Katz, 2 slides
Tosswill and Ridley, 1986	112	Egg in feces	Ritchie
Doenhoff et al., 1993	618	Egg in feces	Kato-Katz, 2 slides
Alarcón-de-Noya et al., 1997	64	Egg in feces	Sedimentation
van Gool et al., 2002	75	Egg in feces	Uunspecified
Sorgho et al., 2005	240	Egg in feces	Kato-Katz, 2 slides
Beck et al., 2008	162	Egg in feces	Kato-Katz, 2 slides
El-Aswad et al., 2011	97	Egg in feces	Unspecified
Stothard et al., 2011	59	Egg in feces	Kato-Katz 2 slides+FLOTAC+Percoll
Kinkel et al., 2012	37	Egg in feces	Unspecified
Smith et al., 2012	12	Egg in feces	Ritchie
Dawson et al., 2013	13 and 24*	Egg in feces	Kato-Katz, 4 slides
Grenfell et al., 2013	20	Egg in feces	Kato-Katz, 18 slides
Sarhan et al., 2014	30	Clinical and epidemiological	None

* 13 for children less than 3 years-old; 24 for children 4 and 5 years-old.

stored reference serum panel is difficult. Therefore, the need for collaborative multicentre projects is important (Banoo et al., 2006), similar to the one reported by Mott and Dixon (1982).

8. Accuracy estimation of serological tests is a problem

An extensive review of serological methods and antibody detection systems for diagnosis of all *Schistosoma* species showed the performance parameters of SEA-ELISAs and included sensitivity ranging from 56 to 100%, and specificity ranging from 6 to 99% (Hinz et al., 2017). After a detailed analysis of the 18 papers selected for the present review, most of which were previously cited by Hinz et al. (2017), we identified and excluded three papers which did not evaluate SEA-ELISAs to detect *S. mansoni* and lacked important data (Fig. 1). The exclusion of these three studies did not change the range of sensitivity and specificity values in the present review, which ranged from 71 to 99% and 6–100%, respectively (Fig. 3 and Table 2). Confidence intervals were also absent in 11/15 reports (Table 2). It should be noted that the number of estimates is higher than the number of reviewed publications because some of the studies tested: (i) more than one diagnostic kit (Kinkel et al., 2012), (ii) different antigen concentrations (Mott and Dixon, 1982), (iii) different test sera dilutions (Sarhan et al., 2014), and (iv) different target populations (Dawson et al., 2013).

As endemic countries move from morbidity control to interruption of transmission, there is a pressing need for more sensitive diagnostics. Ideally, the new tools should conform to the ASSURED criteria: "Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-user" (Kosack et al., 2017; Land et al., 2019). Two of the recommended ASSURED characteristics, sensitivity and specificity, depend on having a rigorous standardization process which includes an evaluation of performance. In addition to new methods to detect antigens, nucleic acids (PCR), and biomarkers, there have been dozens of new diagnostic antigens reported in the literature (Hinz et al., 2017). However, only a few of them are available for populations in need. Moreover, among those few diagnostic antigens which are commercially available, the details of standardization are not disclosed, some are very expensive, and not sufficiently evaluated for application in endemic populations. The failure of many antigens to reach routine use, especially in the last 40 years, deserves more careful study. Two critical issues are standardization of antigen preparations and use of well-established protocols for evaluation of performance. We describe in this review many flaws in standardization, and the effects of these flaws on performance parameters. Furthermore, these flaws may also be present in the standardization and evaluation of other antigens for schistosomiasis, as well as in the diagnosis of other infections. This is an important consideration to address in future studies and should warrant coordinated efforts in order to avoid wasting time and money and implementation failures.

Table 6
Diagnostic methods applied to sera obtained from individuals with parasitic infections other than schistosomiasis in 13 studies evaluating SEA-IgG-ELISA performance between 1979 and 2019.

References	Total number	Methods, infections and their numbers
Grenfell et al., 2013	09	Method: not reported, probably Kato-Katz Hookworm (7); *Enterobius* and hookworm (1); *Trichuris* (1).
McLaren et al., 1979	not reported	Method: Bell filtration *Trichuris* (46); *Ascaris* (35); hookworm (13)
Mott and Dixon, 1982	not reported	Although "presence of other helminthic infection" is mentioned in serum bank data, no detailled data is provided.
Tosswill and Ridley, 1986	15	Method: not described Filariasis (15)
Doenhoff et al., 1993	132	Method: not described *Ascaris* (120); hookworm and *Ascaris* (6); *Trichuris* and *Ascaris* (6)
Alarcón-de-Noya et al., 1997	not reported	Method: Centrifugation hookworm (40%); *Trichuris trichiura* (31%); *Ascaris lumbricoides* (13%)
van Gool et al., 2002	240	Methods: not described fascioliasis hepatica (3); hookworm (10); trichuriasis (16); strongyloidiasis (11); filariasis bancrofti (24); onchocerciasis (12); loiasis (10); hepatic amoebiasis (7); visceral leishmaniasis (5); malaria (9); toxoplasmosis (11); syphilis (8); borreliosis (9); HIV (11); cytomegalovirus (12); Epstein-Barr (12); Hepatite virus A (22); Hepatite virus B (13); rubella (11); Coxsackie B (11); aspergilosis (11)
Sorgho et al., 2005	not reported	The identification of other parasitic infection was recorded but not included in the analysis.
Beck et al., 2008	09	Methods: Hoffman-Pons-Janer and Kato-Katz *Ascaris lumbricoides, Trichuris trichiura* hookworm, numbers not reported
El-Aswad et al., 2011	38	Method: "Microscopic examination" ascariasis (14); *Fasciola hepatica* (3); echinococcosis (3); *Entamoeba histolytica* (13); *Toxoplasma* (5).
Kinkel et al., 2012	84	Methods: "Standard parasitological and serological methods" *Ascaris* (15); Hookworm (3); trichuriasis (9); *Taenia solium* (3); *Trichuris trichiura* and *T. solium* (1); strongyloidiasis (6); fascioliasis (1); filariasis (1); *Hymenolepis nana* (5); *Dicrocoelium dendriticum* (1); cysticercosis (4); echinococcosis (4); *Entamoeba histolytica* (5); giardiasis (5); *E. histolytica* and giardiasis (4); visceral leishmaniasis (6); malaria (11).
Dawson et al., 2013	not reported	not reported
Sarhan et al., 2014	18	Methods: "Parasitological and serology" *Fasciola* (4); hydatidosis (12); Toxoplasmosis (2).

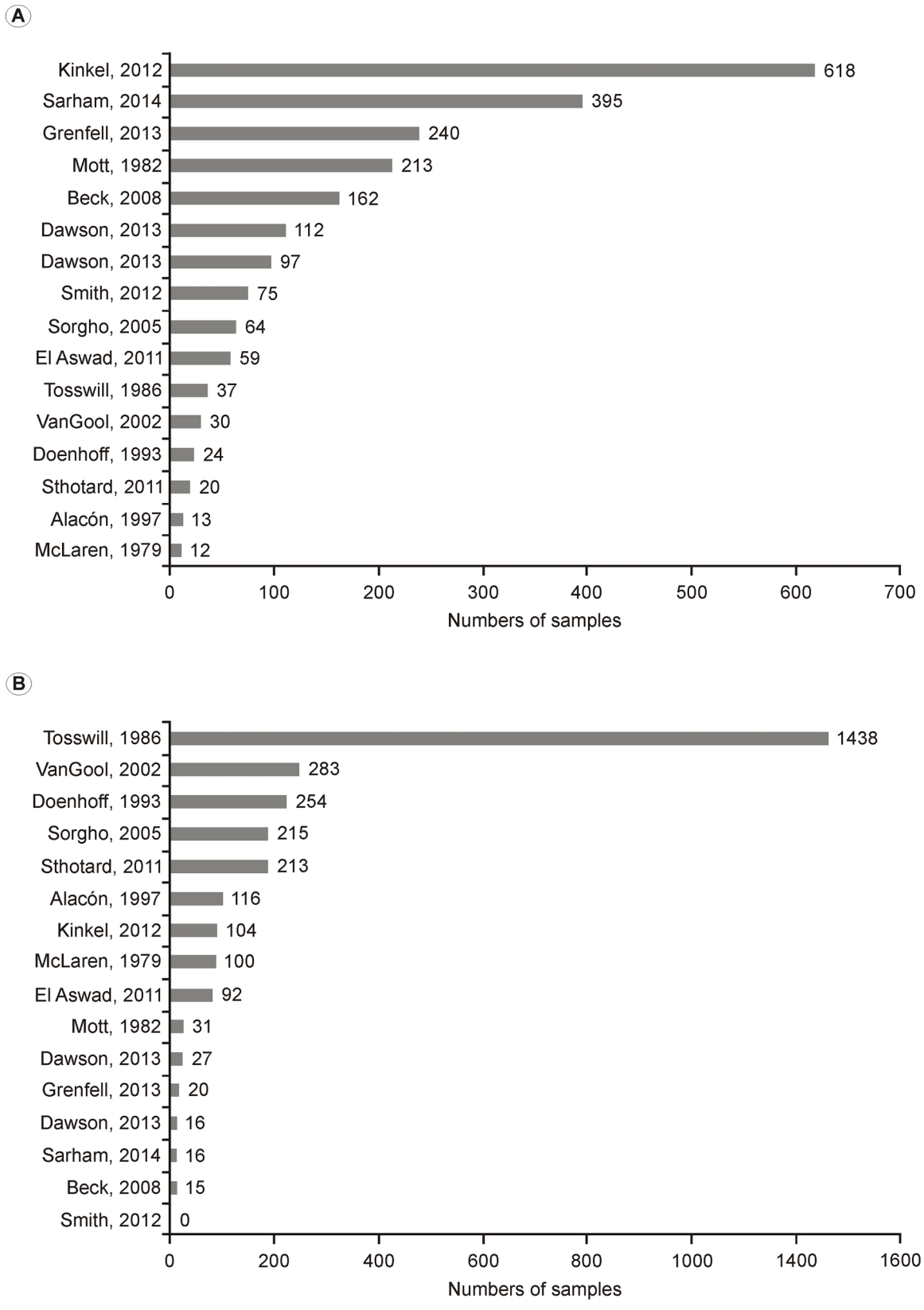

Fig. 2. Distribution of the numbers of positive (A) and negative (B) control serum samples tested in determinations of SEA-IgG-ELISA accuracy for diagnosis of intestinal schistosomiasis. Note the accumulation of values in the lower end of the distributions: 60% (A) and 53% (B) of the numbers are lower than 97, the sample size estimated to provide a comparison of proportions with 80% power and 95% confidence.

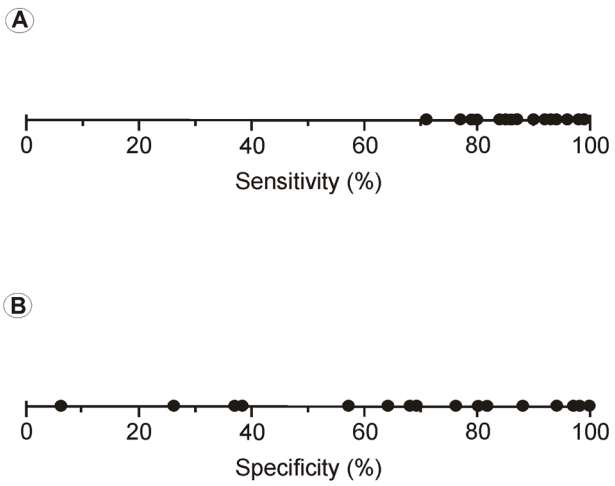

Fig. 3. Distribution of sensitivity (A) and specificity (B) estimates from 15 evaluations of SEA-IgG-ELISA accuracy conducted between 1979 and 2019, for diagnosis of intestinal schistosomiasis.

Detection of antigen in urine with a point-of-care (POC) rapid diagnostic test (RDT) has become increasingly more popular for screening populations in endemic areas than egg detection by the KK method. In particular, collection of urine is much simpler and less expensive than collecting blood or feces (Weerakoon et al., 2015; Utzinger et al., 2015). However, as with other diagnostic systems, antigen detection requires careful standardization and rigorous performance and reproducibility evaluations in several on-site settings. Antigen detection systems may present even higher sensitivity limitations than antibody tests. Contrasting estimates of performance (Oliveira et al., 2018; Lindholz et al., 2018; Peralta and Cavalcanti, 2018; Colley et al., 2020) and reproducibility issues (Viana et al., 2019) should be taken into consideration for better characterization of the diagnostic test. With so many reagents already developed for diagnosis which have not been adequately evaluated, performance evaluation is clearly a process which needs to be improved by the schistosomiasis research community.

9. Perspectives for serology and combinations of diagnostic tests

Considering the complexity of schistosomiasis transmission, it is important to avoid focusing on only one potential diagnostic tool. It is possible that different settings may require different combinations of diagnostic tools (Cavalcanti et al., 2013; Stothard et al., 2014; Siqueira et al., 2016; Al-Shehri et al., 2018). Serology remains an option for diagnosing schistosomiasis. While it may not serve as an initial screening step, in special situations such as clinical settings, it may be used to guide final elimination steps and to certify interruption of transmission (Alarcón-de-Noya et al., 1997; van Gool et al., 2002; Doenhoff et al., 2004; Grenfell et al., 2013; Langley et al., 2014; Sarhan et al., 2014; Ferrera et al., 2020).

10. Concluding remarks

The conclusion reached by Mott and Dixon (1982) almost 40 years ago remains relevant today: conclusive data regarding the best diagnostic antigen for schistosomiasis remains elusive. Our review of the literature for SEA-IgG-ELISA for diagnosis of schistosomiasis, has indicated that many studies lack adequate standardization, efficacy evaluations in laboratories, and proper reporting. We also conclude that our knowledge of SEA-IgG-ELISA accuracy remains incomplete. While the present review focused on one widely used antigen, we suspect that flaws are also present in studies of other antigens and detection systems, and this should be addressed in future reviews. All of the important

Table 7
Proposed components to be described when reporting development and/or evaluation of serological diagnostic methods.

1	Antigen/reagent source and preparation:
	a Strain of the pathogen
	b How the pathogen is maintained and produced in the laboratory (in vivo or in vitro; if in vivo, what hosts are used)
	c Crude or fraction
1-	Intended use of test:
	a Clinical diagnosis
	b Screening at population level
	c Cure control
1-	Sensitization:
	a Description of coupling buffer
	b Description of any physical support used (i.e., polystyrene plate)
	c Protein mass added to each well, tube, bead, or other type of physical support
1-	Criteria for definition of positive control sera
1-	Rationale and methodological details of reference method
1-	Characterization of infection intensity for each serum donor
1-	Criteria for definition of negative control sera (sera with (i) very low or (ii) no reactivity)
1-	Criteria for definition of specificity control sera (sera from individuals infected with other pathogens or having other conditions)
1-	Description of sampling:
	a Strategy for collection: random, convenience
	b Size calculation
1-	Clear definition of cut-off value
1-	Description of sera collection procedure(s)
1-	Origin of samples from specimen banks; authors shall obtain required information from the bank source
1-	Storage conditions for serum samples:
	a temperature
	b conservant addition: sodium azide, glycerol, or other anti-microbial reagent(s)
1-	Dilution of test sera and secondary antibodies
1-	Testing of at least two, and ideally three, serum aliquots
1-	Strategies for reproducibility assessment:
	a Negative and positive control sera included in each experiment, plate, or set of tubes
	b Replicates of serum samples are placed in different rows of plates
1-	Times and temperatures for incubation steps
1-	Blocking strategies: Types and concentrations of blocking reagents
1-	Development:
	a Dilutions of secondary antibodies
	b Description (buffers and concentrations) of the reagents needed for the reaction
	c Reaction stopped at a fixed time or monitored with time (kinetics)
1-	Observers are blinded to other tests results and/or patient identification
1-	Reading the results:
	a Equipment and conditions (i.e., energy spectrum filter)
	b Results expressed as a ratio of serum reactivity to cut-off value
1-	Performance estimates with 95% confidence intervals: sensitivity, specificity, positive predictive value, negative predictive value, accuracy, prevalence
1-	Open access to crude data from testing and demographic data of serum donors
1-	Statement of compliance with:
	a Good laboratory practices (GLP) (WHO, 2001, 2006)
	b Good clinical laboratory practices (GCLP), (WHO, 2009b)
	c Good clinical research practices (GCP), (WHO, 2002)
	d Standard metrology definitions (IOS), (IOS, 2007)
	e Evaluation and reporting (STARD), (Cohen et al., 2016)
	f Desired test characteristics (ASSURED criteria) (Land et al., 2019)
	g Basic definitions, planning and ethical considerations (Banoo et al., 2006)

parameters which describe a test's performance should be considered: sensitivity, specificity, positive predictive value, negative predictive value, and accuracy (Banoo et al., 2006). It is also important to enhance the precision of these estimates by stating confidence intervals (CI) or likelihood ratios (Harper and Reeves, 1999). In the present review, this information was only provided in 4/15 of the publications examined (Table 2). Furthermore, a wide CI should suggest imprecise parameter estimation, and further motivate additional efforts to improve detection methods. As part of quality control procedures, researchers and public health laboratories should comply with International Organization for Standardization (ISO) recommendations for producing estimates of the "uncertainty of measurement (MU) of assay test results" (Dimech et al., 2006).

The ability to collect a panel of well-characterized serum samples is a

significant challenge. Thus, initiatives to establish biobanks which also include other biological materials are urgently needed. A most-welcome initiative is the establishment of specimen banks by the Foundation for Innovative New Diagnostics (FIND), a global non-profit organization and WHO Collaborating Center for Laboratory Strengthening and Diagnostic Technology Evaluation (see https://www.finddx.org).

Both the scientific community and public health personnel need to participate in discussions and improve guidelines for: i) diagnostic target characterization; ii) well-characterized biological material in reference banks; and iii) accuracy of evaluations and reporting. The WHO Special Programme for Research & Training in Tropical Diseases (TDR) and its Diagnostics Evaluation Expert Panel has extensively reviewed the main concepts and basic procedures for evaluating diagnostic methods (Banoo et al., 2006). TDR has published documents about quality assurance and control, like the Good Laboratories Practices Manual (WHO, 2001). If authors also follow the "Standards for the Reporting of Diagnostic accuracy", reporting of results from evaluation studies should greatly improve (STARD-2015; see Cohen et al., 2016). Some of the required characteristics of parasitological and molecular methods may also be included in future STARD revisions. We provide a list of proposed components in Table 7.

Following a demonstration of efficacy, the next necessary step, yet one that is often neglected, is an investigation of the effectiveness, or validation, of studies performed under on-site conditions. This includes the use of proposed tools by active local or regional health workers. On-site settings are also important sources of variability which may not receive adequate attention from researchers (Irwig et al., 2002). Most laboratory investigations on diagnostic antigens are not followed by extensive validation in the field, and are also not incorporated to control interventions in populations affected by schistosomiasis. Bottlenecks that prevent innovative and efficacious solutions from reaching populations in need is the subject of an emerging area of "implementation research" (IR) (Remme et al., 2010; Langley et al., 2014; Krentel et al., 2018). Schistosomiasis control efforts will greatly benefit from IR which is focused on investigations of the difficulties associated with implementing innovative diagnostic solutions. Integration of diagnostic tools for a panel of several neglected tropical diseases in a multiplex platform is also highly recommended (Peeling and Mabey, 2014).

Better standardization and performance evaluations of the many probes investigated within the past four decades are as important as finding new molecules for diagnosis, including antibody targets, ligands to detect antigens, or nucleic acids (WHO, 1993, 2013). By revisiting existing molecules and improving testing procedures, achieving and sustaining the goals of reducing morbidity and eliminating schistosomiasis transmission may be realized.

Declaration of Competing Interest

None.

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Supplementary materials

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