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ORIGINAL ARTICLE

PRELIMINARY RESULTS OF PHAGE DISPLAY METHOD TO PRODUCE RECOMBINANT ANTI-*Schistosoma mansoni* ANTIBODIES

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ABSTRACT

Schistosomiasis is a parasitic disease caused by trematodes of the genus *Schistosoma*. An accurate diagnosis of schistosomiasis is essential for treatment and disease control programs. However, diagnostic techniques may have some sensitivity limitations, especially in mild infections with low egg elimination rates. Therefore, the present study aims to select recombinant antibodies against *S. mansoni* antigens to be applied in the future serodiagnosis of schistosomiasis. Adult parasites of *S. mansoni* were extracted by perfusion of the liver of infected mice. The recombinant antibody methodology was based on phage display, involving three rounds of selection by biopanning. The visualization of the antigen-antibody binding was performed through an indirect ELISA test. The first two selection rounds of biopanning showed positive values for the presence of phages, but the third cycle did not generate a significant amount of phage particles. For the indirect ELISA test, 15 male and 15 female clones were selected. It was observed that most of the antibodies reacted similarly to both male and female antigens. This work has provided valuable information that could be useful for developing diagnostic technologies for Schistosomiasis.

KEY WORDS: Schistosomiasis; recombinant antibodies; biopanning; bioprospecting.

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INTRODUCTION

Schistosomiasis is a parasitic disease caused by trematode parasites of the genus *Schistosoma*. It is a neglected tropical disease, with nearly 240 million people infected worldwide and approximately 700 million at risk of infection (Sady et al., 2015). Transmission has been reported in 78 countries (WHO, 2022), occurring in many developing countries in tropical Africa, the Middle East, Asia, and Latin America (Aula et al., 2021).

It is important to note that schistosomiasis is prevalent in tropical and subtropical areas, particularly in poor communities that lack access to clean water and adequate sanitation. It primarily affects agricultural and fishing populations (WHO, 1999). It is considered the second most important neglected parasitic disease in the world and therefore has a significant impact on public health (de Souza Andrade Filho et al., 2015).

There are at least 25 known species of *Schistosoma* (Pennance et al., 2020), but not all of them are habitual parasites of humans (Colley et al., 2014). There are two major forms of schistosomiasis - urogenital, caused by *S. haematobium*, and intestinal, caused by *S. mansoni* (Wang & Hu, 2014; Mahli et al., 2023). The last one, which was chosen in this study, is the most widely dispersed and is responsible for causing intestinal schistosomiasis, occurring in about 55 countries, including the Arabian Peninsula, Egypt, Libya, Sudan, most Sub-Saharan African countries, some islands in the Caribbean region, Suriname, Venezuela, and Brazil (WHO, 2022). The broad geographical distribution of *S. mansoni* is linked to the widespread distribution of intermediate snail hosts, specifically *Biomphalaria* genus, in the life cycle of this parasite (Morgan et al., 2001).

Laboratory diagnosis of intestinal schistosomiasis is recommended for patients with clinical symptoms and a history of epidemiological exposure. Multiple diagnostic methods are available for detecting the disease. The geographic location may aid in identifying *Schistosoma* species, so it is important to report the area of exposure to the laboratory (Neves, 2019).

The Kato-Katz method is widely used in epidemiological surveys and recommended by the World Health Organization. It involves examining stool samples for the presence of eggs. However, this method has limitations in sensitivity when there is low egg elimination (Speich et al., 2010; Chala, 2023).

Immunological techniques, such as Enzyme-Linked Immunosorbent Assay (ELISA) and point-of-care circulating cathodic antigen (POC-CCA), aim to detect antibodies or antigens. Nonetheless, these techniques may show cross-reactions with other helminths (Neves, 2019). Additionally, molecular techniques like PCR offer high sensitivity and specificity by detecting the parasite's DNA in fecal samples. However, their application may be limited due to the higher cost and complexity (Neves, 2019).

An accurate diagnosis is crucial for improving disease control on multiple fronts. It enables the swift identification of cases, epidemiological surveillance to monitor the prevalence and distribution of the disease, effective planning of targeted control programs, assessment of the effectiveness of implemented interventions, and population education about preventive measures. In summary, diagnosis plays a fundamental role in informing and guiding schistosomiasis control strategies, aiming to reduce its incidence and impact on public health (Weerakoon et al., 2015).

The phage display technique involves presenting recombinant antibodies, in the form of antibody fragments, on the surface of a bacteriophage. This is achieved by inserting a gene encoding the variable regions of antibody fragments into the structural gene of a bacteriophage capsid protein. As a result, the phage particles will display different variable regions of the antibodies on their surface, creating a library of antibody fragments (Gamkrelidze & Dąbrowska, 2014).

These antibody fragments are screened from the libraries using relevant target sites on the surface of the target antigen (Ul Haq et al., 2012). After capturing antigen-specific fragments of interest, they are amplified by infecting an appropriate bacterial strain to express and multiply the phages that express the recombinant antibodies in the form of variable regions of the specific antigen-targeting fragments (Hammers & Stanley, 2014).

Since the vectors used in this technique do not contain a complete genetic code to encode and produce phage particles inside *Escherichia coli*, the M13 helper phage is added and transformed with the library on the phage surface. The M13 provides a set of enzymes and proteins for replication. It is modified by inserting a gene that confers resistance to the antibiotic kanamycin, allowing for preferential growth of cells carrying the phage. Ultimately, the phage particles are extruded through the membrane without causing cell lysis (Sambrook & Russell, 2001). As a result, a library of phages expressing a recombinant monoclonal antibody on their surface (Hammers & Stanley, 2014).

Phage display libraries represent a revolutionary tool for selecting antibodies and are instrumental in exploring protein-protein interactions. These libraries can be constructed with high variability to seek out antibodies with strong affinity for specific antigens. The process of selecting antibodies through phage display entails identifying those with specificity to a target antigen from a vast array of antibody libraries, whether they are immune, non-immune, or synthetic. The ability to screen large collections of phage-displayed antibodies, combined with various selection methods, highlights the growing potential of utilizing antibodies derived from phages as a highly advantageous resource for enhancing the development of therapeutic products (França et al., 2023).

The phage display technique incorporates the process of biopanning, which involves the following phases: a) addition of the phage library to a well

of a plastic microplate where the target antigen is immobilized; b) binding, where phages carrying antibodies with higher affinity bind to the epitopes of the target antigen; c) successive washes to remove nonspecific bindings; d) removal of the selected phages by infecting *E. coli*; e) amplification of the eluted infectious phages in *E. coli* with the help of auxiliary phages, such as MK07 (Hammers & Stanley, 2014; Ribeiro, 2015; Ledsgaard et al., 2018). Typically, these biopanning steps are repeated two or three more times using the amplified phages from the previous cycle of bio-selection to accumulate phages displaying antibody fragments with high affinity (Ledsgaard et al., 2018).

The phage display technique is highly efficient, cost-effective, and easy to apply. It has been widely used as affinity reagents in therapeutics, diagnostics, and biosensors (Tan et al., 2016). Bacteriophage libraries showcasing scFv fragments have demonstrated utility as reagents for identifying antibodies targeting a particular antigen (Sarines et al., 2021).

This technique has already been applied for identifying vaccine candidates using phage display immunoprecipitation followed by a sequencing approach to screen the immune response of infected rhesus macaques during self-cure and challenge-resistant phases (Woellner-Santos et al., 2024). Another example is a study that identified, through the phage display, a recombinant protein (rSjMRP1) that might have the potential for diagnosis of *S. japonicum* infection in domestic animals (Feng et al., 2017).

Therefore, the phage display technique could be a valuable tool in schistosomiasis, especially in the context of diagnosis. By exploring a library of phages displaying peptides or proteins on their surface, it is possible to identify specific antigens of the parasite. These antigens can be used as targets for developing more sensitive and specific diagnostic assays, enabling accurate detection of specific antibodies against *Schistosoma* spp. This promising approach could provide an alternative to conventional diagnostic methods, thus improving the accuracy and effectiveness of schistosomiasis diagnosis (Chen et al., 2004).

As mentioned earlier, existing diagnostic techniques for schistosomiasis may have sensitivity limitations. To address this issue and improve diagnostic accuracy, this study was developed to use libraries of recombinant antibodies for selecting binding molecules against *S. mansoni* antigens. These selecting recombinant antibodies could be evaluated and potentially applied in diagnosing schistosomiasis.

MATERIAL AND METHODS

The present study adopted the parasite *S. mansoni* as the model organism. It was chosen due to its suitability for laboratory maintenance,

as it shows good adaptation to its intermediate host, making it accessible to maintain its biological cycle (Rollinson & Southgate, 1987).

Adult *S. mansoni* parasites (Belo Horizonte strain, Brazil) were obtained from *Mus musculus* mice (CD1 strain), considered a suitable model for studying this parasitic disease. Mice were selected based on their high susceptibility to infection and the ability of the parasite to develop to the adult stage and produce fertile eggs. The parasite life cycle is maintained in the Institute of Hygiene and Tropical Medicine (IHMT) of the NOVA University of Lisbon.

All procedures involving the handling of mice followed animal safety and welfare protocols, were approved by the Laboratory Animal Ethics Committee in accordance with applicable legislation and were based on protocols approved by the General Directorate for Veterinary of the Ministry for Agriculture, Rural Development and Fisheries, Portugal (ID approvals: 023351 and 023355).

DAB, Tomlinson I, and Tomlinson J Libraries

This research used three phage libraries: DAB, Tomlinson I, and Tomlinson J. The DAB library is distributed by Source BioScience, on behalf of the Medical Research Council, United Kingdom. It is a phage library displaying human antibody fragments on its surface. It is constructed in the pR2 vector and contains a repertoire of approximately 3×10^9 clones. It is based on a single variable heavy (VH) chain structure (Lee et al., 2007; Madrid et al., 2020). The Tomlinson I and Tomlinson J libraries were created by the MRC Laboratory of Molecular Biology and the MRC Centre for Protein Engineering in Cambridge, United Kingdom. They are cloned using the pIT2 vector and are also based on a single human VH structure. The Tomlinson I library has a 1.47×10^8 clones repertoire, while the Tomlinson J library has 1.37×10^8 clones (Goletz et al., 2002).

Phage Display

Adult worms were placed in a 24-well plate (Orange Scientific Tissue Culture®, Belgium) containing 10 µL of the three phage libraries described above and Phosphate Buffered Saline (PBS, Sigma-Aldrich®, USA) 0,01M. They were incubated at 37 °C for 1 hour in the Memmert Incubator. Ten washes were performed using PBS-T [supplemented with Tween-20 detergent (AppliChem®, Germany) 0.05% final concentration] to eliminate phages that did not bind to the antigens. Then they were transferred to six Eppendorf tubes and brought into contact with *E. coli* bacteria using the TOP10F strains (MRC HGMP Resource Centre®, Cambridge, United Kingdom) in Luria-Bertani (LB)

medium (Roth®, Germany) and agar (VWR®, Belgium) supplemented with tetracycline (Roth®, Germany) (20 µg/mL) in an orbital shaker (Jepo Tech®, SK-300, Korea) with incubation at 37 °C for 1 hour. After the incubation, the solution was transferred to six 250 mL Erlenmeyer flasks containing 50 mL of LB medium supplemented with ampicillin (Sigma®, USA) (100 µg/mL) and tetracycline (20 µg/mL) and agitated at 37 °C for 1 hour. Glucose (VWR®, Belgium) (1% final concentration) was then added to the flasks, and they were agitated again at 30 °C. Next, LB medium supplemented with ampicillin (100 µg/mL), tetracycline (20 µg/mL), kanamycin (Eurobio®, France) (100 µg/mL) and $MgCl_2$ (5 mM) final concentrations with 1×10^9/ mL MK13 helper phage was added to the same flasks, which were incubated overnight in the incubator to select only *E. coli* cells that were infected by the phage. After 18 h at 30 ºC, the cells were centrifuged at 8,000 × g for 15 minutes, and the supernatant was collected. The phages present in the supernatant were precipitated by adding a PEG solution [20% Glycol polyethylene (PEG 8000] (Roth®, Germany) and 2.5 M NaCl (Merck®, Germany) at 4 °C for 18 hours to concentrate the phages.

The concentration was determined by measuring the absorbance (Abs) using a Nanodrop spectrophotometer (ThermoScientific®). Finally, the phages were stored at -70 °C to maintain their cryoprotection until the next affinity selection step for antibody screening. The biopanning protocol was repeated twice, using PEG-purified phages from the previous selection, diluted 1/10, and used in the subsequent cycle as a library, aiming to obtain a selection of polyclonal antibodies with a high degree of specificity to the target antigen. During each round, phages that did not bind to the antigen were washed away, while those that did bind were eluted and amplified for the next round. This iterative process enriches phages displaying high-affinity monoclonal antibodies. After the final round of biopanning, the enriched phage population was plated on bacterial lawns to form individual colonies, where each one represents a single phage clone displaying a unique antibody fragment.

Visualization of antigen-antibody binding by Indirect ELISA

An ELISA in-house protocol was developed and optimized and consisted of the following steps. Initially, a buffer was made to prepare the antigen, consisting of 0,01M PBS, Glycerol 20% (NZYTech®, Portugal), 10 µM dithiothreitol (DTT, Amresco®, USA), 10 µM phenylmethylsulfonyl fluoride (PMSF, Sigma®, Germany). 400 µL of this solution was transferred to two tubes containing the adult worms, one for females and the other for males. A small amount of silicon carbide was added and macerated using a plastic homogenizer. It was centrifuged for 5 minutes at 8,000 × g. Then, 50 µL of

antigen was added to a flat-bottom 96-well plate (Greiner®) and incubated at 37 °C for 30 minutes. After washing with H_2O, the reaction was blocked for 30 minutes at room temperature with 100 µL of 1% polyvinyl alcohol (PVA, Aldrich®, USA) with 124 – 186 kDa and 98-99 percent hydrolysis (% Hyd). The liquid was discharged, and PBS-T was added. Then, 70 µL of phages were added, diluted ½ in PBS-T with one row on the plate used as a control.

The plate was incubated at 37 °C for 30 minutes, followed by three washes with PBS-T. Next, 50 µL of anti-phage (M13) antibodies with biotin-conjugated (Sigma®, USA) at a dilution of 1/2000 in PBS-T was added, and the plate was incubated at 37 °C for 30 minutes. Three washes with PBS-T were performed, followed by adding 50 µL of streptavidin-AP conjugate diluted 1/5000 in PBS-T supplemented with 0.1% BSA. In the initial experiment, it was observed that the conjugated enzyme exhibited binding affinity to the parasite, indicating potential cross-reactivity that could lead to false-positive results. To mitigate this issue, the conjugate was pre-incubated with an adult male *S. mansoni* worm in 250 µL of PBS-T. So, it was centrifuged and then diluted to the final concentration. The pellet, which contained antibodies exhibiting non-specific binding to the worm, was discarded. This step aimed to enhance the assay's specificity and reduce background noise. The plate was incubated at 37 °C for 30 minutes, followed by four washes with PBS-T and one wash with H_2O. Then, 50 µL of the substrate 4-nitrophenyl phosphate diluted to 1 mg/mL in substrate buffer (100 mM of ethanolamine supplemented with 50 mM of $MgCl_2$) was added, and the plate was incubated at 37 °C for 1 hour. Similar to the studies by Delfosse et al. (2000) and Griep et al. (2000), the reaction was not stopped. The absorbances were measured at 405 nm using a spectrophotometer (Tecan Infinite 200 PRO) to select the recombinant phages with the highest absorbance in ELISA. A plate was prepared for each library, for male and female worms, resulting in six plates with 96 wells each. In the end, the five highest values from each plate were selected.

RESULTS

Biopanning

Only the first and second selection cycles showed positive values for the presence of phages. The third cycle did not generate a significant number of phages, and no pellet formation was observed after the centrifugation of the samples. The virus concentration/ mL is presented in Table 1; it ranged from 0.34 to 1.037 virus/mL × 10^{13} in the first round and from 0.193 to 0.342 virus/ mL × 10^{13} in the second round.

Table 1 - Virus concentration/mL in each Panning 1 and 2 samples.

Panning	Sample	virus/mL × 10^{13}
1	1 - DAB - ♀	1.037
	2 - Tomlinson I - ♀	0.941
	3 - Tomlinson J - ♀	0.8
	4 - DAB - ♂	0.543
	5 - Tomlinson I - ♂	0.34
	6 - Tomlinson J - ♂	0.785
2	1 - DAB - ♀	0.244
	2 - Tomlinson I - ♀	0.221
	3 - Tomlinson J - ♀	0.262
	4 - DAB - ♂	0.316
	5 - Tomlinson I - ♂	0.342
	6 - Tomlinson J - ♂	0.193

Visualization of antigen-antibody binding by Indirect ELISA

Indirect ELISA was performed to determine if the antibodies reacted equally to antigens from both sexes. Negative control was implemented to confirm that observed reactivity is due to the antigen rather than non-specific reagents or contamination. The cut-off value for this ELISA data, based on the given negative control OD values, was 0.1716. This value can be used to determine whether the OD values of test samples are significantly higher than those of the negative controls.

Figures 1 and 2 show the test results conducted with the top five selected colonies from each library based on the highest absorbance values measured by spectrophotometry at a wavelength of 405 nm. The results of the 10 selected colonies and their respective libraries are highlighted. In selecting clones for males, corresponding to the three libraries, DAB had the highest absorbance values, wherein three samples with optical density ranging from 0.22 to 0.25. Tomlinson I and Tomlinson J libraries had one clone presenting optical density with relevant results, respectively, 0.22 and 0.24.

In selecting female clones, according to the three libraries, Tomlinson J had the highest absorbance values, wherein three samples with optical

density ranged from 0.27 e 0.33. DAB and Tomlinson I libraries had one clone presenting optical density with relevant results, respectively, 0.27 e 0.32.

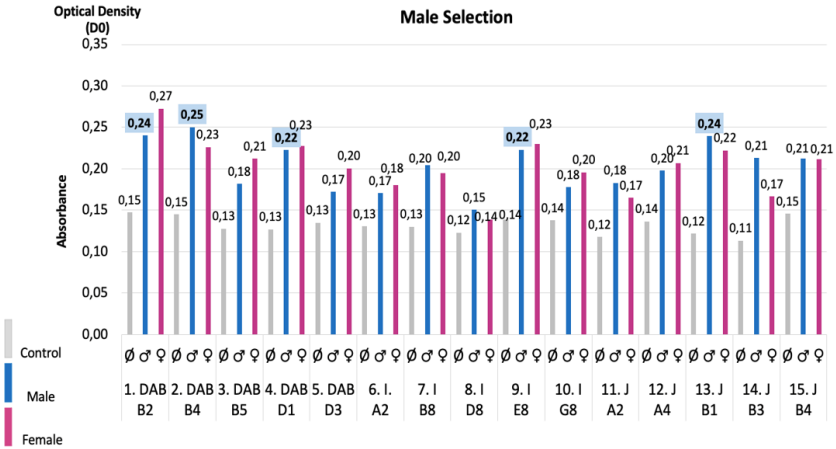

Figure 1. Selection of male clones of *Schistosoma mansoni*, corresponding to the DAB (1 – 5), Tomlinson I (6 - 10), and Tomlinson J (11 - 15) libraries.

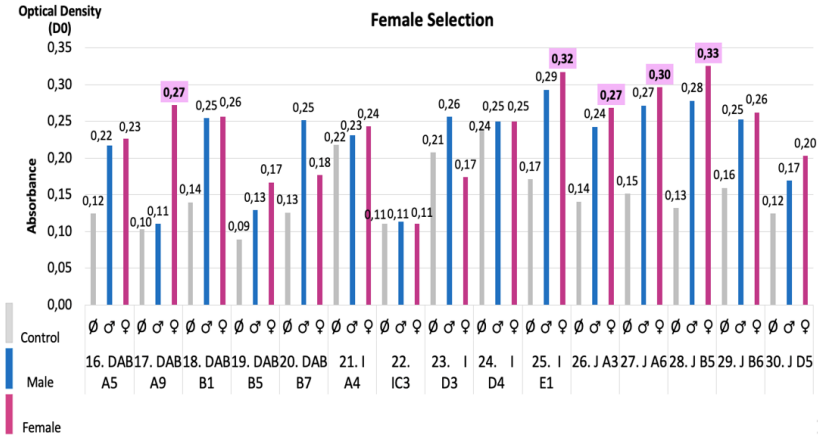

Figure 2 - Selection of female clones of *Schistosoma mansoni*, corresponding to the DAB 16 – 20), Tomlinson I (21 – 25), and Tomlinson J (26 – 30) libraries.

DISCUSSION

At the beginning of the process, unspecific antibody libraries containing unbiased peptide repertoires were used, where millions of phages

were presented in the form of phage particles. The initial selection cycles aimed to enrich phages that recognize a broad array of epitopes on the parasite membrane. This approach was designed to capture the diversity of antigens expressed by *S. mansoni*, ensuring that it does not miss potential diagnostically relevant targets. While the initial selections may yield phages that recognize multiple epitopes, individual phage clones were isolated and screened separately using monoclonal phage ELISA after the final round of biopanning. This allowed us to identify and select the phages that recognize single specific epitopes.

Phage particles were obtained in the first cycle of selection. This indicates that the protocol can isolate clones of interest already in the first selection phase. However, no phage particles were detected in the third round of biopanning in the three scFv libraries used, although it followed the same protocol as in previous cycles. This differs from some studies that were also performed using the phage display technique, where there has been a progressive increase in the concentration of phage particles (Xiaokun, 2018). The results obtained were similar to those observed by Ribeiro (2015), where recombinant antibodies were used for the diagnosis of *Pneumocystis pneumonia* caused by *P. jirovecii*, using the Griffin1, Tomlinson I, and Tomlinson J libraries, the latter two also applied in the present study.

The fact that phage particles were not detected in the last selection cycle may be related to the washes performed to remove non-specific bindings. During the biopanning process, it is possible that some phage particles may have been lost during the washing steps (Mandecki et al., 1995). This risk exists, and this bias can usually be corrected by adjusting the number of washes and the interval between them.

The absence of satisfactory results in the third round may indicate that specific antigens of the parasite may not have been properly captured or displayed by the phages during the biopanning process. It suggests the need to review the biopanning strategy. This may involve optimizing experimental conditions, such as adjusting the selection and amplification conditions of the phages, modifying the phage library, or selecting new targets for the next round of biopanning.

Notably, this study exclusively utilized antigens derived from adult worms, specifically proteins located on the schistosome's outer surface (tegument). An examination of *S. japonicum* tegument proteins revealed antigens highly reactive to patient sera. Among these, STIP1 stood out as a particularly immunoreactive tegument protein, exhibiting strong antigenicity and thus presenting itself as a promising biomarker for diagnosis (Chen et al., 2014).

In a separate investigation also about *S. japonicum*, a membrane protein (Sj23HD) emerged as a significant early diagnostic indicator when compared to Soluble Egg Antigen (SEA) (Wang et al., 2011). A subtegumental protein

(SjCypA) has also been identified as another potential diagnostic candidate (Han et al., 2012).

Possibly, there are tegument or membrane *S. mansoni* proteins, which are equally reactive and have potential for use in diagnosis. But this needs to be further studied. The aquaporin, for example, was identified as the most abundant transmembrane protein in the *S. mansoni* tegument through proteomic studies and could also serve as a viable target for diagnostic purposes (Castro-Borges et al., 2011).

Although *S. mansoni* SEA is recommended to detect and increase the sensitivity of diagnostic tests, tegument antigens are also an option since the tegument is the main interface between the parasite and the host, and these antigens can play a crucial role in the host's immune response. Adult worms express a diverse array of proteins, many of which are also present in other life stages and can be released into the host's circulation. The possibility of identifying antigens present in the tegument, which could be detected early during infection, could allow for faster and more effective diagnosis.

The ELISA technique confirmed that the antigens were binding with some antibodies. Most of the antibodies reacted with parasites from both sexes, suggesting that males and females share similar antigens. It is important to say that total antigens were used in this test, including proteins extracted from the adult worms of *S. mansoni*. The protocol for antigen obtention can promote the loss of conformational epitopes due to the parasite maceration process. In the study by Sarhan et al. (2014), the total antigens ELISA was analyzed comparing egg antigens, cercarial, and soluble adult worms, using sera of schistosomiasis patients in different concentrations. They concluded that for the diagnosis of *S. mansoni*, total antigen reactivity revealed the best sensitivity by soluble adult worms.

The literature suggests that the absence of specific bindings may be related to four reasons: (1) the presence of toxic sequences that can destroy specific antigen-phage bindings during the amplification and purification of phage particles (Hoogenboom et al., 1998); (2) contamination during bacterial manipulation (Jeong et al., 2010); (3) the use of heterologous hosts that have restricted codons or regulatory expression, leading to the non-expression of fragments with specific nucleotide sequences and no binding to the target antigen (Gustafsson et al., 2004; Green & Goldman, 2021); (4) incomplete fusion between the fragment and the protein when transferring the vector to a bacterial strain, resulting in non-fused fragments without a complete structure (Lee et al., 2007).

Another issue worth discussing is the choice of the blocking agent. Some authors suggest that one of the most suitable agents is PBS-T (Green & Goldman, 2021). In an indirect ELISA assay conducted by Ribeiro, 2015, during research, other blocking agents such as 1% BSA and 1% gelatin were tested, but only PBS-T showed absorbance readings on the spectrophotometer.

However, the PVA used in the present study would have superior blocking capacity compared to commonly used blocking agents such as skimmed milk (Lim et al., 2013). Furthermore, research has analyzed the blocking ability of PVA in enzyme immunoassays by coating wells with PVA of different MW and % Hyd. They concluded that PVA with 124 – 186 kDa and >99 % Hyd, similar to PVA used in this study, was the most effective in suppressing the binding of the conjugate (Rodda & Yamazaki, 2009).

Another point that should be mentioned is that other methods did not test the binding of the antibodies selected. The antibodies were also not tested with antigens from other *Schistosoma* spp. or other parasites. Notably, the antigens came from the surface of the entire adult worm, used in biopanning, and from the macerated adult worm, used in the indirect ELISA. Antibodies have not been tested with other specific antigens. Additional steps must be taken to guarantee that it will have a unique phage displaying a monoclonal antibody after selection. This includes subjecting phage clones that show specific binding to DNA sequencing to determine the genetic sequence of the displayed antibody fragment.

All these issues may have been a limitation in the results of the present study. However, this can be justified because this study is a pilot project; therefore, many protocols must be adjusted and optimized.

This work generated information that may be useful for the development of technologies for the diagnosis of Schistosomiasis. This study pioneered applying recombinant antibodies for detecting *S. mansoni* antigens using the phage display technique. It was possible to obtain antibody fragments that reacted to both male and female antigens similarly.

Antigens identified through phage display can be incorporated into immunological tests, such as ELISA, to detect the presence of specific antibodies against the schistosomiasis parasite in patients' serum. These tests can be more sensitive and specific than conventional diagnostic methods. By identifying antigens recognized by the host's immune system during the initial phases of infection, the phage display technique may enable early detection of schistosomiasis, allowing treatment to begin and the prevention of complications associated with the disease.

However, it is suggested that future research adopt measures to correct or minimize the biases encountered. It is important for the genetic sequencing of the samples to confirm the whole sequence of the light and heavy chains of the scFv. Furthermore, using the selected recombinant antibodies for immunological assays (ELISA) to detect the parasite in human biological samples would be necessary. Also, it should be tested with other specific antigens, either from *S. mansoni* itself, different species of *Schistosoma*, or even other groups of parasites. Finally, it is suggested that the binding of the selected antibodies should also be demonstrated by another method, such as

western blotting or immunofluorescence tests, which were not employed in the present study due to the study's schedule.

In conclusion, despite the limited results, this pilot study provides an initial foundation for developing diagnostic assays using *S. mansoni* in the phage display technique. With further optimizations and proper validation, these assays could serve as effective tools in the early and accurate diagnosis of intestinal schistosomiasis. The preliminary results of this study underscore the need for protocol refinement and additional validation but also offer prospects for developing new approaches in disease diagnosis using the phage display technique.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest to disclose.

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