OXFORD

BIOLOGY Methods & Protocols

Biology Methods and Protocols, 2023, bpad006

https://doi.org/10.1093/biomethods/bpad006

Advance Access Publication Date: 3 April 2023

Review

UCP-LF and other assay methods for schistosome circulating anodic antigen between 1978 and 2022

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Abstract

Detection of circulating anodic antigen (CAA) is known for its high sensitivity in diagnosing schistosomiasis infection, even in low-prevalence settings. The Up-Converting Phosphor-Lateral Flow (UCP-LF) assay developed in 2008 presented greater sensitivity than other assay methods in use for CAA detection. Our study aims to comprehensively review all studies conducted in this area and thus generate informed conclusions on the potential for adopting the UCP-LF assay for diagnosing this important yet neglected tropical disease. Using the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, we generated search criteria to capture all studies in English journals available in the Scopus and PubMed databases on 20 December 2022. A total of 219 articles were identified, and 84 that met the inclusion criteria were retrieved and eventually included in the study. Twelve different assay methods were identified with a noteworthy transition from enzyme-linked immunosorbent assay (ELISA) to the UCP-LF assay, a laboratory-based assay that may be applicable as a point-of-care (POC) diagnostic test for schistosomiasis. Reducing the time, cost, and dependence on specialized laboratory skills and equipment, especially relating to the trichloroacetic acid extraction step and centrifugation in the UCP-LF CAA assay may go a long way to aid its potential as a POC tool. We also propose the development of a CAA-specific aptamer (short protein/antigen-binding oligonucleotide) as a possible alternative to monoclonal antibodies in the assay. UCP-LF has great potential for POC application.

Keywords: UCP-LF; assay-methods; schistosomiasis; circulating-anodic-antigen; review

Introduction

Schistosomiasis is a snail-borne parasitic disease affecting more than 240 million individuals globally [1]. It is one of the most neglected tropical diseases caused by the trematodes genus *Schistosoma*. The disease is contracted when cercariae (Schistosome free-swimming larvae) penetrate the skin of persons exposed to infected freshwater. The early stage of schistosomiasis, characterized by dermatitis in the region where the cercariae penetrate the human host, is one of the major clinical presentations of the disease [2]. Clinical manifestations in this phase (the acute systemic phase) are caused by the schistosomula (immature worms) migrating through the heart and lungs to the vasculature of the liver, where the parasite will reside and mature [3]. When infected, the blood-dwelling flukes known as schistosomes, digenetic trematodes (flatworms) reside in the hosts vasculature and lay eggs from which the bulk of infectious illness symptoms ensue [3]. There are over 20 different species of schistosome. Three of these have the greatest impact on public health; *Schistosoma haematobium*, which causes urogenital schistosomiasis, *Schistosoma mansoni*, which causes intestinal schistosomiasis; and *Schistosoma japonicum*, which is the primary cause of hepato-intestinal schistosomiasis. *Schistosoma mekongi* and *Schistosoma intercalatum* are equally significant medically although less widespread and geographically limited to a few regions.

The lack of proper diagnostic procedures has significant implications for the epidemiology of the disease [4, 5]. For effective control of schistosomiasis, new or improved detection approaches are necessary, especially in nonendemic areas [6]. Diagnostic techniques for schistosomiasis fall into three broad categories: parasitological detection (such as the Kato-Katz (KK) method); serology, which includes antibody (Ab)- and antigen-detection; and molecular assays (such as detection of circulating nucleic acid) [7]. The first-line approach for schistosomiasis diagnosis is the examination of the stool of the infected individual (the KK method). However, its sensitivity is insufficient at low

Received: January 26, 2023. **Revised:** March 27, 2023. **Accepted:** March 31, 2023

infection and prevalence [8]. Microscopic detection of eggs in the host's stool (*Schistosoma mansoni*, *Schistosoma japonicum*) or urine (*Schistosoma haematobium*) remains the classical diagnostic measure for schistosomiasis and is unsuitable for early diagnosis (acute infection) [9]. Ab-based serological assay and detection of Schistosoma DNA in the serum of infected persons have the major drawback of being unsuitable for monitoring treatment efficacy and specifying active infection. Individuals remain DNA positive in serum post-treatment [10]. Hence there is a need for a diagnostic biomarker that disappears after treatment but can be detected accurately in active infection from the early stages.

Schistosomes actively secrete and excrete particular antigens into the host circulation at different developmental stages. Based on these stages, crude antigens are classified as cercarial antigens, adult worm-associated antigens (e.g. tegument or gut-associated) and egg antigens. Most important among these are two gut-associated circulating antigens, which are regurgitated into the host circulation from the Schistosome's gut by adult and young worms. These circulating antigens are the subject of most research among these groups, acting as biomarkers of active schistosomiasis. These are circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) [8, 11]. A major limitation is the inconsistency of the point-of-care (POC)-CCA as a diagnostic tool in areas of low endemicity and its significant false-positive rate [12]. Much recent diagnostic research is now focused on the CAA, whose major antigenic character arises from its novel polysaccharide, which has been reported to be a highly specific diagnostic marker in schistosomiasis [13]. Schistosoma CAA is mostly assessed in serum and urine by enzyme-linked immunosorbent assay (ELISA) or LF assays employing monoclonal antibodies (mAb). However, several advancements have been made in the assay techniques used to detect and quantify the antigen.

Consequently, this study was designed to systematically identify all the methods that have been developed or applied in the detection and/or quantification of the CAA as a biomarker in the diagnosis of schistosomiasis or to monitor responses to its treatment. We will focus on the Up-Converting Phosphor-LF (UCP-LF) assay, which is a test with ultimate sensitivity used in several studies to validate active infection. Adapting the UCP-LF assay in POC diagnosis would greatly aid screening for schistosomiasis and monitoring treatment onsite. We, therefore, highlight the application of aptamer, which could be a step toward making the assay in a POC format.

Materials and methods

Search strategies

An all-inclusive literature search of published articles on the detection/quantification of CAA as a diagnostic biomarker of schistosomiasis was conducted systematically on PubMed and Scopus databases up to 23 December 2022. The following search terms were used: "circulating anodic antigen", "CAA", "schistosome", "schistosomiasis", "schistosoma", "detection", "diagnosis" and "diagnostics". Boolean operators, AND/OR/NOT, combined the search terms (Table 1). We limited the search to peer-reviewed articles that were published in the English language. The paper selection process followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 guideline [4, 14].

Inclusion criteria and data extraction

The following inclusion criteria were applied for articles to be retrieved: (i) original research reporting data on Schistosoma CCA and (ii) original research reporting diagnostics detection/quantification of the antigen. Review articles, meta-analyses, and studies without full text were excluded and studies focusing on CCA instead of CAA. The data extraction format from the reviewed papers included the year of publication, assay method, assay conditions/principles, and overall conclusion of the assay in detecting CAA.

Results

Search results

The search identified 219 articles, comprising 119 from Scopus, which were exported in BibTeX format and 100 articles from PubMed, also exported in BibTeX via TeXMed—a BibTeX interface for PubMed (https://www.bioinformatics.org/texmed/). Both files were merged and tidied together with 89 duplicates removed, on: https://flamingtempura.github.io/bibtex-tidy/. Two articles written in Chinese were removed, and then the remaining 128 records were screened by the titles and abstracts, from which 27 were excluded for not meeting the inclusion criteria. We eventually evaluated 101 articles per the eligibility criteria and excluded 17 more. Finally, 84 articles included in the study met the eligibility criteria and quality assessment (Fig. 1).

Summary of detection/quantification techniques for CAA

Articles reviewed in this study ranged from 1978 to 2022 (Fig. 2). There was an overall transition observed from the use of different forms of serological or antigen/Ab-related assay methods (1978–2008) to the use of UCP-LF assay (2008–22) for the detection/quantification of CAA (Figs. 3 and 4). Earlier serological assays identified made use of immunoprecipitation by the defined antigen substrate spheres (DASS) system, immunoelectrophoresis (IEP), indirect haemagglutination assay (IHA), immunofluorescent assay (IFA), double Immunogold labeling (IGL) assay, magnetic bead antigen capture enzyme-linked immunosorbent assay (MBAC-EIA), ELISA, time-resolved immunofluorometric assay (TR-IFMA), and mAb-based antigen-capture ELISA (Table 2). The development of the UCP-LF assay (Table 3) by Corstjens *et al.* [15] was a major breakthrough in the quantification of CAA, and it enhances improved diagnosis of active schistosomiasis and monitoring of therapeutics.

UCP-LF CAA assay

Conventionally, to perform the UCP-LF assay, trichloroacetic acid (TCA) is first used to extract CAA from the clinical sample to be analyzed (either urine, serum, plasma, or whole blood) [15]. During the extraction process, protein material is precipitated, and immune complexes resulting from antigen-Ab reactions will be separated, whereas CAA and other carbohydrates are left in the solution. Centrifugation can be carried out to obtain *TCA-sup* (a clear supernatant that contains the CAA). Anti-CAA mAb (mAb-CAA) can then be conjugated to the luminescent UCP reporter particle and then introduced into the *TCA-sup*. An optional mixing and incubation of the resulting mixture for 1 h at 900 rpm and 37°C are sometimes carried out to improve the assay sensitivity when the 400 nm yttrium orthosilicate (which is a type of upconverting phosphor material that is commonly used in biomedical imaging and diagnostics) particles are used. The incubation can be omitted when using smaller yttrium fluoride particles (100 nm). For further improvement of the sensitivity, Amicon filtration devices can be used to concentrate the TCA-sup. This allows an increase in the volume of the sample [69]. LF strips

Table 1: Search strategies

Database	Search strategy
Scopus	TITLE-ABS-KEY ("circulating anodic antigen" OR caa AND schistosom* AND detection OR diagnos*) AND (EXCLUDE (DOCTYPE, "re") OR EXCLUDE (DOCTYPE, "cp") OR EXCLUDE (DOCTYPE, "le") OR EXCLUDE (DOCTYPE, "sh"))
PubMed	("circulating anodic antigen"[Title/Abstract] OR caa[Title/Abstract]) AND (schistosom*[Title/Abstract])) AND (detection[Title/Abstract] OR diagnos*[Title/Abstract]) NOT (review[Publication Type])

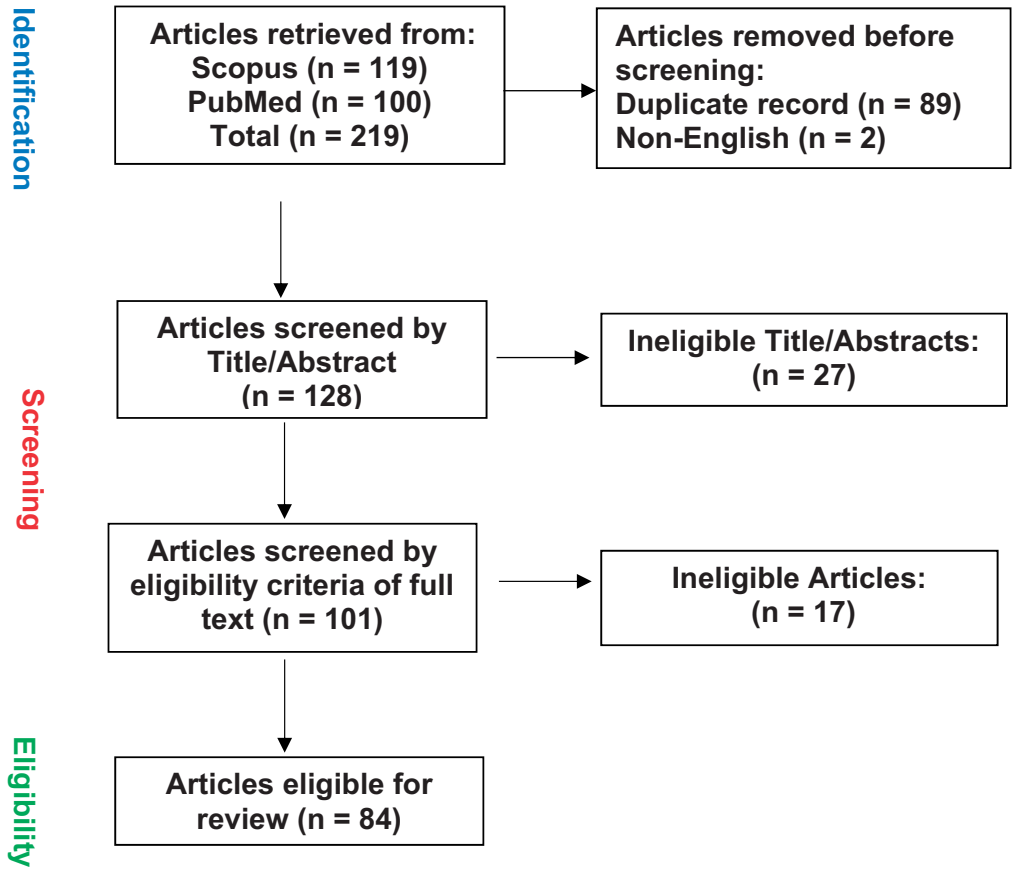

Figure 1: PRISMA study selection flow chart.

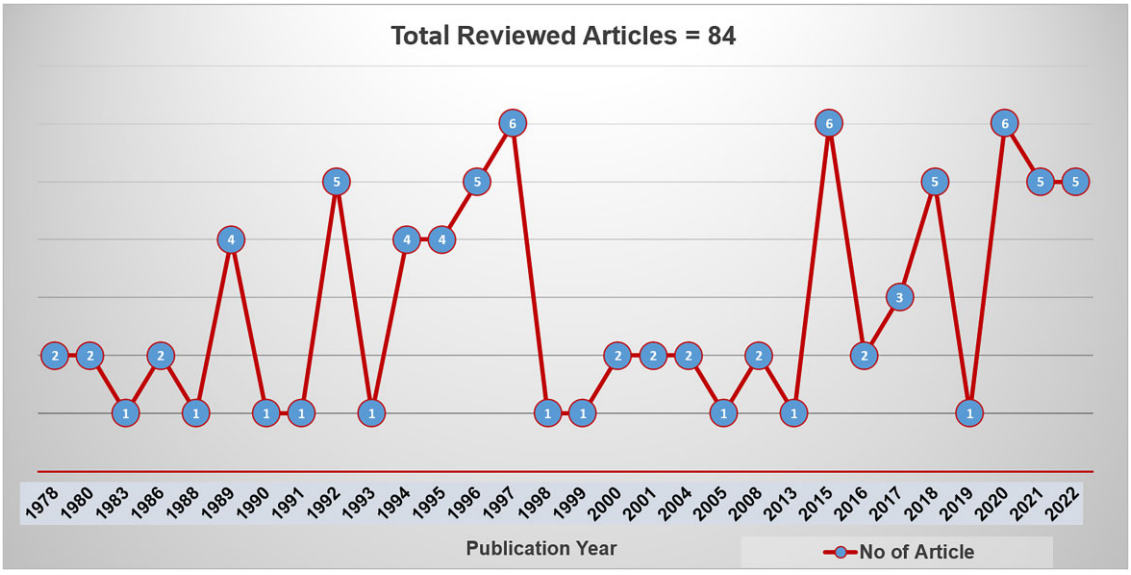

Figure 2: Research productivity on detection and quantification techniques of CAA reviewed.

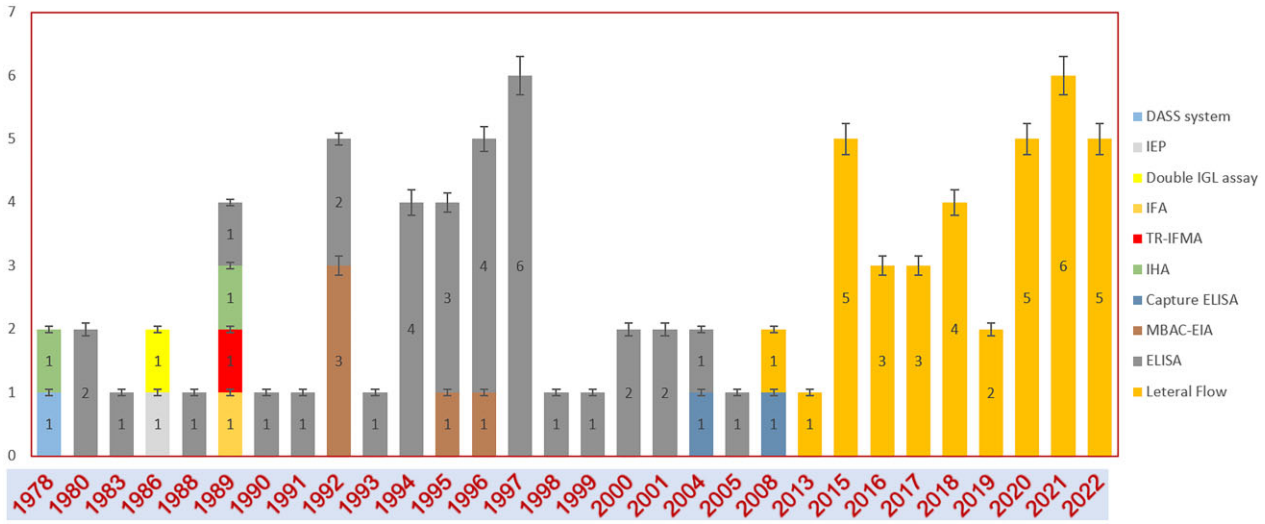

Figure 3: Trend analysis of the analytical assay methods on detection/quantification of CAA.

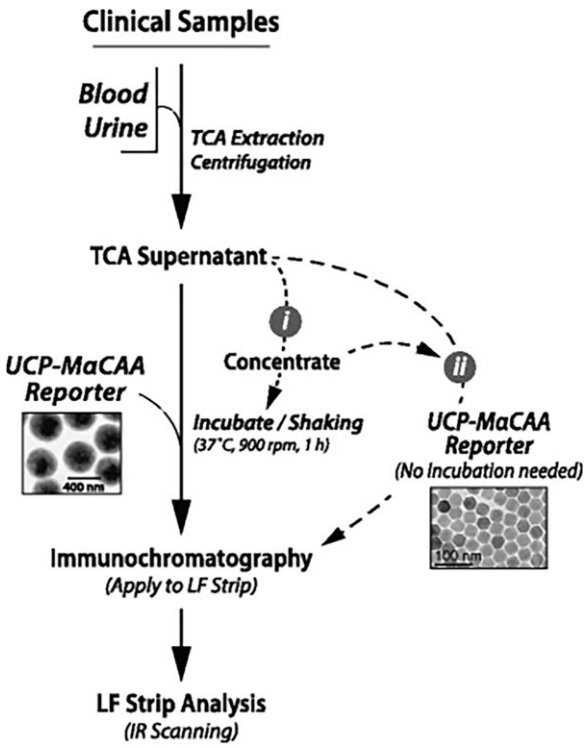

Figure 4: UCP-LF assay schematic flow on detection and quantification of CAA.

comprising a mAb-CAA test line are used to examine the incubated mixture by immunochromatography [15]. Specialized strip readers are required to scan the LF strip for UCP reporter signals to quantify the CAA content of the analyzed sample [15]. Figure 4 shows the schematic representation of the UCP-LF CAA assay, where UCP-MαCAA is a Luminescent UCP reporter coated with mouse mAb-CAA [72].

Discussion

The detection of the genus-specific Schistosoma CAA has proven to be effective both for the diagnosis of schistosomiasis and for monitoring treatment efficacy. The major switch in CAA assay is from the ELISA-based test to an LF-based one, demonstrating a higher sensitivity of about 10-fold higher than the CAA-ELISA [15]. The UCP-LF assay entails a sample pretreatment step, where the component of the antigen is extracted with TCA and is centrifuged to obtain the TCA-sup, containing the targeted CAA carbohydrate structure, which can be analyzed on an LF strip [15] utilizing a unique luminescent reporter. These UCP particles were developed to enhance the assay's sensitivity [94]. The UCP reporter technology involves the excitation of the luminescent reporter particles with infrared light (IR, 980 nm) to emit higher energy green light (550 nm) in a process called up-conversion. This process is completely restricted to the particle lattice and is thus free of auto-fluorescence from other assay components (as detailed in Corstjens *et al.* [95]). UCP-LF for CAA involves using the same mA to bind the antigen to both the test (T) line on the LF strip and the UCP reporter, the antigen being sandwiched comprises a repetitive structure. The CAA is still detectable after storing the clinical samples at ambient temperature for prolonged periods or after repeated freezing and thawing [68], owing to the stability of the carbohydrate component in urine and blood samples. The CAA carbohydrate structure (containing repeating GalNAC and GlcA disaccharides) is unique [13], and no biological equivalent has been described elsewhere.

The UCP-LF assay for CAA detection was later adapted to a dry reagent format that improves ease of storage at ambient temperature and shipment across different parts of the world [64, 69]. Corstjens *et al.* [95] proposed further improvement of the sensitivity of the UCP-LF CAA assay by including a concentration step in the sample pretreatment, which was demonstrated by Corstjens *et al.* [69]. This step involves the concentration of the TCA-soluble fraction of urine samples, allowing more considerable sample input, increasing the volume from 10 µl of urine to 7500 µl. The results confirm that the larger sample volume identified samples with CAA concentrations well below the 30 pg/ml cutoff threshold for the standard dry-reagent UCP-LF CAA assay without concentration and requiring only 10 µl urine (UCAA10) [95]. A typical example is the sample set tested in the study of [69], where 2000 µl urine concentration assay (UCAA2000) improved CAA detection sensitivity from 30 pg/ml to 0.3 pg/ml. In general, 10 µl of a urine sample (UCAA10 assay) may be sufficient in high endemic areas to correctly indicate the occurrence of schistosomiasis by individual

Table 2: Earlier serological assays used to detect or quantify CAA

Methods	Principle of operation	Studies Reference	No
DASS system	Using a specific Ab to precipitate a particular protein/antigen out of the solution.	[16]	1
IEP	Protein components of a mixture are first separated by electrophoresis, and then a mixture of antibodies specific for the antigens is added to a trough cut in an agar. The individual antigens and their specific antibodies will diffuse toward one another, and lines of precipitate form by their interactions is being analyzed.	[17]	1
Double IGL assay	Detects 2 antigens (CAA and CCA). Double labeling is done with the two kinds of primary antibodies after being mixed in a single solution.	[18]	1
IFA	The use of fluorescent-labeled antibodies for the specific detection of antigens.	[19]	1
TR-IFMA	Detection of an antigen of interest by using both mAb-coated microtiter wells and nanoparticle chelate-labeled mAb together	[20]	1
IHA	The antigen is adsorbed onto glutaraldehyde-fixed red blood cells to detect Ab specific to the antigen in a serum sample by specifically targeting the particular antigens and binding tightly to them.	[21, 22]	2
MAb-based antigen-capture ELISA	mAb-coated microtiter wells and a nanoparticle chelate-labeled mAb are used together for the specific detection of antigens.	[23, 24]	2
MBAC-EIA	Magnetic beads were used as the Ab carriers to improve the assay sensitivity and shorten the reaction time.	[25–28]	5
ELISA	Conjugating an Ab to an enzyme before using it to identify its specific antigen, which will be detected as the enzyme converts its substrate into an observable end product. The substrate may be either a fluorogenic or a chromogen.	[29–63]	35

Table 3: LF assays used to detect or quantify CAA

Methods	Principle of operation	Study Reference	No
Up-converting Phosphor Technology-Based Lateral Flow (UPT-LF)/UCP-LF assay	The same mAb is used to bind (sandwich) the antigen of interest to both the UCP reporter and the specific capture area of the mAb-containing test (T) line of a lateral flow strip. The immune interaction on the strip will then be analyzed based on the UCP reporter signals.	[2, 10, 11, 12, 15, 64–91]	33
Poly(amidoamine)-coated magnetic particles-Lateral flow Assay	poly(amidoamine)-coated magnetic particles are being utilized to concentrate the antigen from a large volume of the clinical sample before the UCP-LF assay	[92]	1
UCNP-LF	Several photon-upconverting nanoparticles (UCNPs), having the ability to emit light of shorter wavelengths under near-infrared (near-IR) excitation which hardly interacting with biological materials, are being applied to the lateral flow assay as reporter particles	[93]	1

diagnosis, whereas the UCAA250 assay is ideal for the follow-up to mass drug administration at the individual level. However, in low endemic settings, the most single-worm infection can be detected with the UCAA2000 assay for individual diagnosis [69]. Despite the outstanding sensitivity of UPC-LF CAA, it requires substantial time, laboratory skills, and equipment that limits it from being applicable as a POC assay. To this effect, Markwalter *et al.* [92] developed an alternative method that does not depend on laboratory apparatus (i.e. centrifugation) for concentrating the antigen from the large volume of urine samples. The technique utilizes poly (amidoamine)-coated magnetic particles, which have positively charged dendrimers that electrostatically attract the highly negatively charged CAA. When the antigen is captured on the surface of the magnetic beads, the supernatant can be removed. A high salt elution buffer (compatible with UCP-LF) could be used to concentrate the CAA into a small volume. This approach yielded a potential 200-fold enhancement in CAA detection [92] as compared to a UCAA10 assay.

Limitations

Only two databases were consulted for the articles analyzed. Also, other assay methods aside the UCP-LF have not been fully reviewed in this work.

Conclusion

The UCP-LF CAA is the assay with the highest sensitivity for application in schistosomiasis diagnosis. Its potential for adoption as a POC test in its current format is limited as it is laboratory-based and requires centrifugation equipment. This susceptible format is also time-consuming and costly as it involves the use of Amicon concentration devices. However, the UCP-LF CAA assay is genus specific and can detect Schistosoma species in either serum or urine. Developing and applying CAA-specific aptamer as an alternative to CAA-specific mAs in combination with ultrasensitive detection platforms could result in further

improvement in schistosomiasis diagnosis without compromising the sensitivity of the UCP-LF assay technique. Aptamers have many advantages over antibodies, including smaller size, equal specificity and affinity to the target, better stability, easier immobilization and modification and higher reproducibility.

Availability of data and materials

The datasets/information used for this study is available from the corresponding authors upon reasonable request.

Acknowledgements

Not applicable.

Authors' contributions

Ilemobayo Fasogbon (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Writing—original draft [lead]), Patrick Aja (Project administration [equal], Supervision [equal], Writing—review and editing [equal]), Erick Ondari (Supervision [equal], Validation [supporting], Writing—review and editing [equal]), Ismail Adebayo (Supervision [equal], Writing—review and editing [equal]), Olukayode Ibitoye (Data curation [equal], Methodology [equal]), Moses Egesa (Investigation [equal], Writing—review and editing [equal]), Tusubira Deusdedit (Supervision [equal], Writing—review and editing [equal]), S. Sasikumar (Investigation [equal], Writing—review and editing [equal]), Hope Onohuean [Conceptualization [equal], Formal analysis [equal], Methodology [equal], Resources [equal], Software [equal], Validation [equal], Writing—review and editing [equal]).

Funding

Not applicable.

Conflict of interest statement. None declared.

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