STUDY PROTOCOL

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Prospective, observational study to assess the performance of CAA measurement as a diagnostic tool for the detection of *Schistosoma haematobium* infections in pregnant women and their child in Lambaréné, Gabon: study protocol of the freeBILy clinical trial in Gabon

Yabo Josiane Honkpehedji[1,2,3], Ayôla Akim Adegnika[1,2,3,4], Jean Claude Dejon-Agobe[1,2,5], Jeannot Fréjus Zinsou[1,2], Romuald Beh Mba[1], Jacob Gerstenberg[1,2], Raphaël Rakotozandrindrainy[6], Rivo Andry Rakotoarivelo[7], Tahinamandranto Rasamoelina[8], Elisa Sicuri[9], Norbert G. Schwarz[10], Paul L. A. M. Corstjens[11], Pytsje T. Hoekstra[3], Govert J. van Dam[3], Andrea Kreidenweiss[2,11*] and on behalf of the freeBILy Consortium

Abstract

Background: *Schistosoma* antigen detection in urine is a valuable diagnostic approach for schistosomiasis control programmes because of the higher sensitivity compared to parasitological methods and preferred sampling of urine over stool. Highly accurate diagnostics are important in low *Schistosoma* transmission areas. Pregnant women and young children could particularly benefit from antigen testing as praziquantel (PZQ) can be given to only confirmed *Schistosoma* cases. This prevents the unborn baby from unnecessary exposure to PZQ. We present here the protocol of a diagnostic study that forms part of the freeBILy project. The aim is to evaluate the accuracy of circulating anodic antigen (CAA) detection for diagnosis of *Schistosoma haematobium* infections in pregnant women and to validate CAA as an endpoint measure for anti-*Schistosoma* drug efficacy. The study will also investigate *Schistosoma* infections in infants.

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* Correspondence: andrea.kreidenweiss@uni-tuebingen.de
[2]Institut für Tropenmedizin, Universität Tübingen, Tübingen, Germany
[11]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, the Netherlands
Full list of author information is available at the end of the article

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Methods: A set of three interlinked prospective, observational studies is conducted in Gabon. The upconverting phosphor lateral flow (UCP-LF) CAA test is the index diagnostic test that will be evaluated. The core trial, sub-study A, comprehensively evaluates the accuracy of the UCP-LF CAA urine test against a set of other *Schistosoma* diagnostics in a cross-sectional trial design. Women positive for *S. haematobium* will proceed with sub-study B and will be randomised to receive PZQ treatment immediately or after delivery followed by weekly sample collection. This approach includes comparative monitoring of CAA levels following PZQ intake and will also contribute further data for safety of PZQ administration during pregnancy. Sub-study C is a longitudinal study to determine the incidence of *S. haematobium* infection as well as the age for first infection in life-time.

Discussion: The freeBILy trial in Gabon will generate a comprehensive set of data on the accuracy of the UCP-LF CAA test for the detection of *S. haematobium* infection in pregnant women and newborn babies and for the use of CAA as a marker to determine PZQ efficacy. Furthermore, incidence of *Schistosoma* infection in infants will be reported. Using the ultrasensitive diagnostics, this information will be highly relevant for *Schistosoma* prevalence monitoring by national control programs as well as for the development of medicaments and vaccines.

Trial registration: The registration number of this study is NCT03779347 (clinicaltrials.gov, date of registration: 19 December 2018).

Keywords: Schistosomiasis, *Schistosoma haematobium*, Clinical trial, UCP-LF CAA, *Schistosoma* circulating antigen, Pregnancy, Infant, Diagnostic test, Praziquantel, Gabon

Background

Schistosomiasis is one of most widespread human parasitic diseases worldwide [1, 2]. Infections with blood flukes of the genus *Schistosoma* are endemic in 74 countries with more than 230 million individuals being infected and 90% of infections are occurring in sub-Saharan Africa [3]. *Schistosoma haematobium* is highly prevalent in Africa [4] resulting in chronic morbidities, particularly affecting urogenital organs. Women in child-bearing age suffer two-fold from *Schistosoma* infections: directly from immune-pathological effects induced by worm infection, and indirectly from potential adverse birth outcomes during pregnancy [5].

Currently, there is no vaccine available against schistosomiasis and control is based on a single drug - praziquantel (PZQ) for chemotherapy and prevention. The 2012 WHO roadmap on neglected tropical diseases calling for schistosomiasis elimination initiated a tremendous increase in PZQ deployment [6]. This fuelled the ever-growing concern of emergence of PZQ resistant *Schistosoma*, particularly since schistosomes with reduced susceptibility have been identified sporadically in sub-Saharan Africa [7–10]. However, drug efficacy outcomes largely depend on the accuracy of the surrogate endpoint measures that reflect treatment effects in clinical trials [11]. Clinical evaluation of PZQ efficacy, of new PZQ formulations (e.g. paediatric) [12] or of new drug candidates relies on reliable diagnostic instruments to correctly judge a drug's potency to kill *Schistosoma* infections in humans. Egg microscopy is still the reference standard widely used in schistosomiasis diagnosis but the methodology lacks sensitivity and cannot precisely measure drug efficacy due to huge variation in egg

counts intrinsically associated with egg excretion that varies from day-to-day and occurs in clusters in stool [13–17]. Egg reduction rates vary considerably in a study cohort necessitating a huge sample size in clinical trials to be able to identify significant differences between the investigational product and the control. Furthermore, available PZQ efficacy data may be limited [18, 19].

Pregnant women and their infants are two vulnerable population groups, particularly in sub-Saharan Africa, who - amongst other infectious agents - are heavily exposed to *Schistosoma* infections. Infants were thought not to be exposed enough to get infected in their first months of life, however meanwhile high infection rates in infants aged as young as 3 months have been demonstrated [20]. *Schistosoma* infection causes nutritional and hematologic deficits and overt pathology caused by chronic inflammation to eggs trapped in the bladder and urogenital tissues and can lead to granulomatosis and progress to fibrosis and cancer at the late stage. Approximately 20 million women are suffering from female genital schistosomiasis (FGS) which is caused by embolized eggs in tissues of the uterus, cervix and the lower genital tract associated with bleeding and pain. FGS is a significant risk factor for HIV infection [21, 22].

Detrimental effects of maternal schistosomiasis during pregnancy on the foetus may lead to poor birth outcomes including low birth weight, preterm delivery and maternal anaemia [5, 23, 24]. Schistosomiasis is a large contributor to anaemia and undernutrition and is per se unfavourable for the developing foetus. In 2002, the WHO recommended that all pregnant women positive for *Schistosoma* should be treated with PZQ either individually or as part of mass drug administration (MDA)

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programs during the second and third trimester [25, 26]. Adoption of the recommendation and implementation by national disease control programs was however delayed in most African countries, due to the side-effects of PZQ and the lack of safety data in pregnant women and unborn babies. First results from randomised controlled trials with PZQ in pregnancy meanwhile have provided evidence for the safety of PZQ also in newborns [27, 28]. Nonetheless, pregnant women, unborn babies and newborns are vulnerable populations and there is a reluctancy to include them in PZQ (mass) treatment campaigns as this would imply unnecessary exposure to PZQ (even of pregnant women and infants that are not infected). In addition, logistical and financial obstacles have delayed implementation of PZQ preventive chemotherapy in pregnant women at country levels. Accordingly, innovative patient-centred approaches, combining accurate schistosomiasis diagnosis and targeted treatment during pregnancy, are needed to control schistosomiasis in this population.

Rationale for the study

In Gabon, *S. haematobium* is the major prevalent *Schistosoma* species and *S. intercalatum* [29] is present only at a minimal level. According to the recommended WHO strategy, in 2015 a total of 343.554 individuals would have required preventive schistosomiasis treatment in Gabon alone [30]. Despite being an upper middle-income country [31], there is no effective national control program for schistosomiasis established in Gabon, but rather the National Program for neglected tropical diseases (NTD) which includes schistosomiasis and other neglected diseases. A recently published study indicated that approximately 10% of the pregnant women living in Lambaréné and Fougamou vicinities tested positive for *S. haematobium* infection with increased odds (OR1.7, 95% CI 1.03–2.82) for low birth weights of the newborns [32, 33]. As it is true for most of the observational and interventional studies on schistosomiasis, the power of the study was weakened due to the low sensitivity of the reference schistosomiasis diagnosis applied (egg microscopy), and one might assume that a considerable proportion in the control group were misclassified as negative.

Diagnostic tests that are highly sensitive and specific are essential for the detection of *Schistosoma* infections and are urgently needed in a variety of applications such as a test-and-treat strategy to control schistosomiasis in pregnancy and as tools to determine efficacy of new interventions in clinical trials. Detection of *Schistosoma* circulating antigens is becoming an important *Schistosoma* diagnostic strategy. Circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) are constantly released by schistosomes into the host circulation

and day to day fluctuation of CAA in serum is relatively little [34]. Furthermore, CAA levels are correlating with the number of worms [35, 36]. As CAA and CCA have been shown to clear within a few days or weeks after PZQ treatment [18, 34, 37], assays measuring antigen levels are promising techniques to more accurately assess drug efficacy. Particularly, the detection of circulating anodic antigen (CAA) by the up-converting phosphor-based lateral flow (UCP-LF) technology has proven to be a highly sensitive and specific diagnostic tool for schistosomiasis [37–39]. The high sensitivity and specificity of the UCP-LF CAA test is warranted by the uniqueness of the antigen to the *Schistosoma* genus, in combination with specific, high affinity monoclonal antibodies, a carbohydrate specific sample pre-treatment step and a unique background free reporter technology [37, 40, 41].

The overall aim of this study is to evaluate the accuracy of the UCP-LF CAA urine test for the detection of *S. haematobium* infections in pregnant women and to validate CAA as an endpoint measure for PZQ efficacy. This study is part of the *Fast and Reliable Easy-to-Use Diagnostics for Eliminating Bilharzia in Young Children and Mothers* (freeBILy) project which aims to thoroughly evaluate the use of CCA and CAA antigen tests for the diagnosis of *Schistosoma* infections in pregnant women and their newborns [42]. The freeBILy project is complemented by an additional trial performed in Madagascar that investigates a CCA-based test-and-treat strategy integrated into routine maternal and child primary health care programmes.

Methods

Study design

The freeBILy Gabon-study consists of a set of 3 interlinked prospective, observational sub-studies (A-C), each targeted to assess a specific objective (see Fig. 1 for a schematic trial design, procedures and stages).

Sub-study A is a prospective, cross-sectional, observational study conducted in 1000 pregnant women in Lambaréné and surroundings to determine the sensitivity and specificity of the UCP-LF CAA test (index test) for the detection of *S. haematobium* infections in urine samples. Sampling will include standardized urine collection on 3 consecutive days for *S. haematobium* detection by egg microscopy and UCP-LF CAA testing; qPCR and POC-CCA will be done on one urine sample. To control for concomitant parasite infections one stool sample (within the 3 days of urine sampling) will be collected to screen for *S. intercalatum* and soil-transmitted helminths infections, as well as one blood sample will be taken for filaria and *Plasmodia* spp. detection. The blood sample will also be used to measure anti-*Schistosoma* antibodies and to provide blood cell counts and
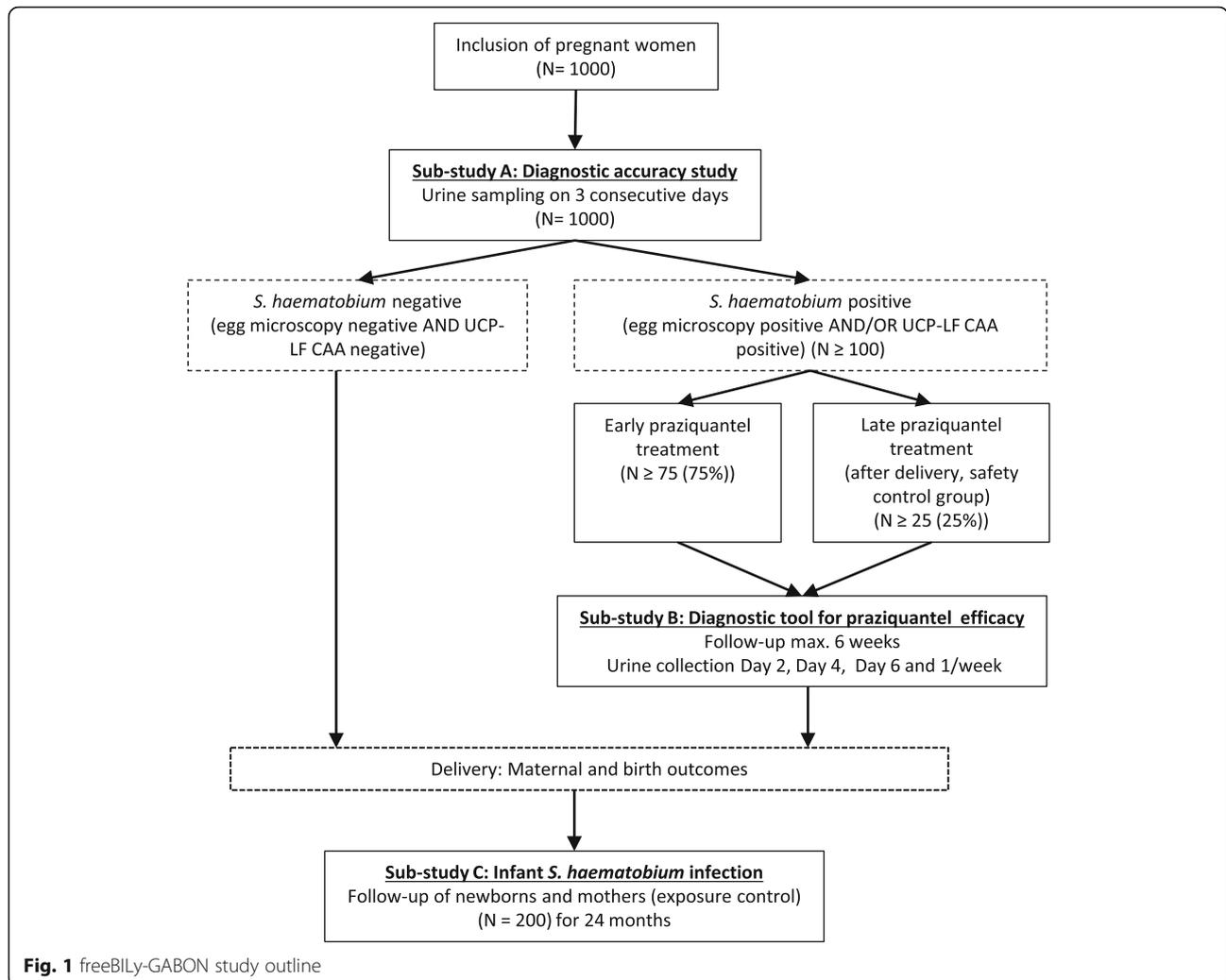
Fig. 1 freeBILy-GABON study outline

haemoglobin levels. The primary objective of sub-study A is to compare the UCP-LF CAA urine test against a composite diagnostic reference including egg microscopy, qPCR, and serology in order to determine UCP-LF CAA test performance. Sub-study A will allow to identify participants for sub-study B.

Sub-study B is an observational, follow-up study of at least 100 pregnant women identified in sub-study A as being infected with *S. haematobium* (by egg microscopy and/or urine UCP-LF CAA test). *Schistosoma*-infected women will be randomised 3:1 (75:25) for immediate PZQ treatment during the pregnancy (single dose of 40 mg/kg) or delayed PZQ treatment after delivery (control group). The control group will not be treated during pregnancy (to monitor "natural" CAA level fluctuation in a participant if not treated), but 6 months after delivery. The primary objective is to determine the kinetics of CAA after treatment during pregnancy compared to the control group. The control group serves also as a safety control of PZQ administration to pregnant women and

exposure of their offspring (s). Therefore, both groups (treatment and control) will be actively followed-up and urine will be collected on day 2, day 4 and day 6 after PZQ treatment and then once a week until both egg microscopy as well UCP-LF CAA assay become negative but no longer than 6 weeks after PZQ treatment. The secondary objective is to collect data on safety of PZQ during pregnancy. For details on the safety investigation, see below (treatment, adverse events and safety assessment).

Sub-study C will follow up infants born to mothers included in sub-study A and their mothers for a maximum of 24 months ($N = 200$). This is an observational, longitudinal study with the primary objective to determine the incidence of *S. haematobium* infection and age of first *S. haematobium* infection in infants. Therefore, after delivery, mother and her infant will be asked to provide urine every 3 months until the UCP-LF CAA test becomes positive for *S. haematobium* in the infant. Children who test *S. haematobium* positive will be

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treated with praziquantel according to Gabonese national treatment guidelines.

In addition, a customized questionnaire addressing the knowledge, attitude and practice (KAP) of schistosomiasis of mothers and infants will be asked. Health-related quality of life (HRQoL) will also be measured by using a standardized tool: this will allow filling a wide literature gap, where only very few studies have attempted to measure HRQoL in schistosomiasis endemic areas [43]. To our knowledge, this study will be the first one collecting information on HRQoL among pregnant women in areas endemic of schistosomiasis and of other parasitic infections such as malaria.

Study site and population
The study is planned to take place in two areas located in the center of Gabon: Lambaréné including vicinities (Bindo, Makouké and Nzilé-PK villages) and Fougamou. Both regions are endemic for *S. haematobium* with the prevalence ranging from 7 to 75%, depending on the area or the population age group [32, 44–46]. The study population are pregnant women and their newborns.

Ethical considerations
The study protocol and informed consent forms (ICF) were approved by the National Ethics Committee of Gabon (PROT N°039/2018/SG/CNE) and by the Institutional Ethics Committee at CERMEL/Gabon (N° 017/2018). Written informed consent will be sought from any volunteer or the legal guardian of minor volunteers before any study procedure will be performed.

Recruitment of participants, data and sample collection
Prior to enrolment, the study will be explained to the pregnant women – and legal guardian if minor - presenting to the antenatal care centres (ANC) in Lambaréné (Albert Schweitzer and Regional Georges Rawiri Hospitals). Those willing to participate will be provided with the informed consent form (ICF) for signature for themselves and for their unborn child. Particular emphasis is given to information on *Schistosoma* transmission, diagnosis, associated pathologies and risk factors for *Schistosoma* infection in particular, and to intestinal helminths in general. Inclusion criteria are pregnancy at gestational age between 16 and 30 weeks (based on last date of menses), providing signed ICF, willing to deliver in one of the two maternities in the study areas and no plans to move out of the study area during the following 24 months. Exclusion criteria are reporting of complicated previous pregnancy, known chronic infections or diseases as HIV, HBV, HBC infections. Following the ICF process, a KAP questionnaire will be administered to collect standard demographic, health history, obstetrical data and information on schistosomiasis related

knowledge, attitude and practice.. Economic and health-related quality of life (HRQoL) questionnaire will be included as well. The questionnaire will be used to measure HRQoL among all pregnant women at enrollment and to monitor HRQoL among the positive women over time. For this, the HRQoL questionnaire will be administered again to positive women 1 week after enrollment when test results are available and treatment provided (at A4) and a few months later, well after treatment completion (at C2). Physical examination will be done by the study physician; participants will be asked to provide urine (midstream urine will be collected in a clean container), stool and blood samples for parasite diagnostics and health care service. At delivery, newborns will be weighted, and gestation age will be recorded. The newborn's anthropometry parameters, Apgar score at 1 and 5 min, maternal bleeding volume, placenta quality and weight will be collected. For details on study visits over time with respect to the study phases and details of sample collection see Additional file 1.

Diagnostics
Investigational product: UCP-LF CAA test
The index test is the UCP-LF strip test detecting and quantifying CAA levels. LF strips can be analysed with POC care readers [47] or, as used in this study, with a multistrip benchtop reader (Upcon; Labrox Oy, Turku, Finland) capable of analysing 20 strips at a time. The UCP-LF CAA test was developed at Leiden University Medical Center (Leiden, The Netherlands) [38, 48]. So far, the tests are produced in-house at LUMC in a batch-wise manner (ca. 2000 tests per batch), fully quality-controlled using standardized reference samples, ensuring specificity and sensitivity. A dry reagent format is available that can be stored and transported at ambient temperature [39]. Here, 417 µl of fresh or stored (– 20 °C) urine will be tested by the UCP-LF CAA urine test following published procedures [48] including a concentration step (centrifugal Amicon Ultra 0.5 mL filters with a 10 kD cut-off; Merck Millipore) after pretreatment of the urine with 1/6 volume of 12% trichloroacetic acid and (UCAA*hT*417 format) [49]. All measurements will include a set of CAA-spiked standards into a negative urine to determine the threshold of positivity that is set to ≥2 pg/ml.

Comparator diagnostics
Urine filtration and egg microscopy
Schistosoma egg detection by microscopy is commonly used for the diagnosis of schistosomiasis as recommended by WHO and diagnostic procedures will follow standardized protocols [50]. Urine (10 ml) will be passed through a 12 µm filter (Whatman Nucleopore) using a syringe. The filter will then be placed on a glass slide

and read under a light microscopy (10x magnification). Every slide will be read by two independent microscopists. The test will be considered positive if at least one *S. haematobium* egg is found and egg counts will be given (N eggs/10 ml urine).

qPCR
Schistosoma egg DNA will be detected by real-time PCR (qPCR) on urine samples following a published protocol [51]. We will use the established *Schistosoma* genus-specific primers (Ssp48F and Ssp124R) and the dual labelled probe Ssp78T to amplify a 77-bp fragment of the internal transcribed spacer-2 (ITS2) sub-unit. An internal control will be added to monitor efficient DNA extraction. DNA extraction from urine and qPCR assay will be performed.

Serology
Antibodies against *S. haematobium* will be measured using a lateral flow assay (UCP-LF antibody assay) that is based on the same technology as the UCP-LF CAA test and can be evaluated using the same UCP Labrox reader. The serology test is designed to detect antibodies against soluble egg antigen (SEA) and soluble crude cercarial antigen preparation (SCAP) in sera of participants [37, 52].

Additional diagnostics
POC-CCA
The point-of-care (POC) CCA urine test is a commercially available POC rapid test for the detection of CCA in urine (Rapid Medical Diagnostics, South Africa). This LF immuno-chromatography test delivers qualitative detection of active *Schistosoma* infection, and is recommended and generally applied to detect *S. mansoni* infections performing less in *S. haematobium* settings [53, 54]. It is ideally suited for field conditions as it does not require laboratory facilities or highly trained personnel.

Microscopy for concomitant parasite infections
To control for the impact of concomitant other parasite infections on the accuracy of UCP-LF CAA, stool samples will be investigated for *S. intercalatum* (as well as any other *Schistosoma* species) and for other highly prevalent helminths such as *Ascaris lumbricoides*, *Trichuris trichuria*, Hookworms, and *Strongyloides stercoralis*. Stools will be examined by Kato-Katz and coproculture techniques [53, 55]. Therefore, approximately 50 mg of fresh stool will be processed. Microscopy reading (10x magnification) is done by two independent readers and numbers of eggs per gram of stool will be reported according to WHO guidelines [53, 56]. Remaining stool will be stored for later confirmation by respective qPCR [51]. Blood samples will be

examined for infections with malaria parasites (*Plasmodium falciparum*, non-falciparum) and filarial parasites (*Loa loa*, *Mansonella perstans*) by standard diagnostic procedures (Giemsa-stained thick blood smears, saponin leucoconcentration assay followed by microscopy, respectively) [57, 58].

Treatment, adverse events and safety assessment
Women infected with *Schistosoma* (positive by egg microscopy and/or UCP-LF CAA) will be treated with PZQ either during pregnancy (as recommended by WHO) or after delivery. Single dose PZQ (40 mg/kg) will be administered by an unblinded member of the study team under observation. Safety of PZQ administration in women will be assessed verbally within 24 h (after 1 h and 24 h later) to record all adverse events occurring after drug administration. All adverse events will be assessed by the study physician, following local guidelines and will be graded as mild, moderate or severe. All investigators (study physicians and lab technicians) will be blinded to the treatment allocation. Other parasitic infections will be treated following national treatment guidelines. This work will be done in collaboration with the Gabonese National Program for the Fight against NTDs who will contribute to the PZQ supply.

Additional outcomes
Maternal health outcomes, maternal anaemia (defined as Hb < 11 g/dl) at inclusion and at delivery will be investigated. In offspring, birthweight (with low birthweight defined as weight at birth < 2.5 kg) and small for gestational age (used as an indicator for possible intrauterine growth restriction) will be investigated. In addition, the exposure to PZQ during pregnancy will be evaluated in infants at the age of 12 and 24 months. Time to sit, crawl, stand and walk, incidence of illnesses and vaccine coverage during the follow-up will be recorded.

Data safety and monitoring board
An independent data safety and monitoring board (DSMB) has been established which will review safety data in the sub-study B during the study.

Sample size calculation
Analysis of the UCP-LF CAA (UCAA*h*T417) test performance will be compared against a composite diagnostic reference (i.e. by latent class analysis). Assuming a 10% egg positivity rate in the study population [32], about 1000 pregnant women should be tested to obtain 100 *Schistosoma* positive women. Assuming further a sensitivity of the index test (UCP-LF CAA) between 80 and 100% with 100 infected women, statistical precision (two-sided 95% confidence limit) of ±7.8% will be achieved if a sensitivity of 80% is observed and a lower

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limit of 96.4% if a sensitivity of 100% is observed. Due to the limited sensitivity of the egg detection test, to estimate the specificity, only study participants negative for reference tests should be included. Furthermore, assuming a true *Schistosoma* prevalence of 20% (based on a conservative estimate of 50% sensitivity of the egg test) and assuming a false positive rate of 20% of the other two reference tests (PCR and UCP-LF antibody test), at least 500 women will enter the naïve estimate of specificity. This will lead to a statistical precision of between ± 3.5 and ± 4.3% depending on the observed specificity. For sub-study B, the analysis is based on the assumption of a non-inferiority delta of 0.10, identical probabilities of positivity and negativity at the end of the sub-study B for both tests (UCP-LF CAA and egg microscopy) and a discordance of 12%, with 100 pregnant women, the power will be 80% to show non-inferiority (type 1 error: 2.5% one-sided).

Data management and statistical analysis

All data will be collected on paper forms which will be entered into a REDCap database [59]. Initial data will be double entered in REDCap to ensure their accuracy. Each participant included into the study will receive a unique identifier. The case report from (CRF) is the source documents for personal data, clinical data and laboratory result sheets will be considered as source documents for laboratory results reported in the CRF.

Analysis of the UCP-LF CAA (UCAAhT417) test performance will be compared against a composite diagnostic reference by using Latent Class Analysis or other Bayesian approaches which will allow to compare the estimated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the accuracy values for detecting *Schistosoma* in the sample. The model will be fitted to determine the Bayesian Information Criteria (BIC), Akaike information Criteria (AIC), the Chi square test and the standardized Residuals.

To assess the effect of PZQ treatment on birth weight, Analysis of covariance (ANCOVA) will be used. This is a fundamental statistical method of analysis for both randomised and non-randomised studies. It is a regression analysis which includes parameter estimates for observed covariates in addition to the treatment effects we are ultimately interested in estimating.

Discussion

There is no vaccine against schistosomiasis so far, and MDA of PZQ remains important for the control of schistosomiasis but is not sufficient when moving towards the goal of schistosomiasis elimination. The ideal test for test and treat strategies would be an easy to use point of care test, which is highly sensitive and specific for all *Schistosoma* species (without the necessity to distinguish between the species as the treatment is the same for all species) and used material that does not require invasive procedures (e.g. urine or saliva instead of blood). Microscopic methods lack sensitivity and require a lot of resources time and trained personnel. Serology cannot distinguish between an acute, ongoing and past infection and PCR methods require advanced microbiological facilities and capabilities. Antigen tests, however can provide easy to use, field suitable tests [19, 60, 61]. There is a need of sensitive and accurate *Schistosoma* diagnostics to support communities in need and also control programs. The freeBILy Gabon study will comprehensively evaluate the performance of the UCP-LF CAA laboratory test with the focus on pregnant women and its extended usefulness as a read-out measure for PZQ efficacy.

The UCP-LF-CAA test is highly sensitive and specific and also performs well for urogenital schistosomiasis. Unfortunately, this test is not currently available as a point of care test. The assessment of this test in pregnant women in a *S. haematobium* endemic regions with weekly follow up of initially positive pregnant women as well as in infants to validate the CAA antigen test as an endpoint for measuring PZQ efficacy. Determining the true efficacy of PZQ against different *Schistosoma* infections is important for schistosomiasis control programs. It informs on the susceptibility of local *Schistosoma* infections towards PZQ as well as providing information for future efficacy trials for paediatric formulations of PZQ. Furthermore, using the UCP-LF-CAA test minimizes sample size in future efficacy trials as CAA levels are relatively constant in serum or urine, whereas the number of eggs is subjected to highly random daily fluctuations. The UCP-LF-CAA test clearly demonstrates that prevalence of active *Schistosoma* infections is underestimated by a factor of up to ten when compared to egg microscopy. Especially in low-endemic settings, the UCP-LF-CAA test is superior over parasitological methods. This test can easily identify individuals with very low levels of infection as well as early infections and is well-suited for determining efficacy (or even failure) of treatment. The test is ready to be taken up by industry for upscaling and deployment at large scale. So far, there is no alternative for evaluating long-term MDA programmes, and moving towards post-MDA surveillance or elimination.

Beyond the study, this work will prepare for the development of CAA rapid diagnostic tests, that can play a role in the future as a test-based schistosomiasis treatment strategy in pregnancy in endemic regions. Furthermore, the study tackles a neglected disease (schistosomiasis) and provides a concept to overcome the neglect of pregnant women and small children by schistosomiasis control programmes.

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Trial status

Recruitment of study participants has started in April 2019 and is expected to be finished by April 2021: Follow-up of sub-study B is expected to be finished by July 2021 and of sub-Study C by the end of 2022.

Supplementary information

Additional file 1. Overview of study visits over time with respect to the study phase and details of sample collection, describes the study phases and procedures.

Abbreviations

ANC: Antenatal care; CAA: Circulating anodic antigen; CCA: Circulating cathodic antigen; CERMEL: Centre de Recherches Médicales de Lambaréné; CRF: Case report form; FGS: Female genital schistosomiasis; GA: Gestational age; Hb: Hemoglobin; HIV: Human Immunodeficiency Virus; LUMC: Leiden University Medical Center; MDA: Mass drug administration; NTD: Neglected Tropical Disease; POC-CCA: Point-Of-Care Circulating Cathodic Antigen; PZQ: Praziquantel; qPCR: (Semi-)quantitative real-time polymerase chain reaction; UCP-LF: Up-converting phosphor technology based lateral flow; WHO: World Health Organisation

Authors' contributions

Drafted the manuscript: YJH; revised the manuscript: AK, YJH, JCDA, JFZ, RBM, JG, RR, RAR, TR, ES, PLAMC, PTH, GJvD, NGS, ES, AK, AAA; Conceptualized and designed the study: AK, AAA; Drafted the study protocol: AK, YJH, JCDA, JFZ, AAA; Conceptualized the freeBILy project: GJvD, PTH, AK, NGS; Obtained grant funding to conduct the work: RAR, TM, ES, NGS, AK, AAA, PTH, GJvD; Development and supervision of *Schistosoma* antigen testing: PLAMC, PTH, GJvD; All authors have read and approved the manuscript.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study (Study Protocol Version 1, 27 June 2018) was reviewed and approved by the National Ethics Committee of Gabon (PROT N°039/2018/SG/CNE) and by the Institutional Ethics Committee at CERMEL/Gabon (N° 017/2018). Only participants given written informed consent (or the legal representative if minor) are enrolled in the study. The Centre de Recherche Médicale de Lambaréné, Gabon, has the trial's sponsorship.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Author details

[1]Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon. [2]Institut für Tropenmedizin, Universität Tübingen, Tübingen, Germany. [3]Department of Parasitology, Leiden University Medical Center, Leiden, the Netherlands. [4]German Center for Infection Research (DZIF), Tübingen, Germany. [5]Department of Infectious Diseases, Division of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. [6]Université d'Antananarivo, Antananarivo, Madagascar. [7]Université de Fianarantsoa, Fianarantsoa, Madagascar. [8]Centre d'Infectiologie Charles Mérieux, Antananarivo, Madagascar. [9]Fundación Privada Instituto de Salud Global Barcelona, Barcelona, Spain. [10]Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany. [11]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, the Netherlands.

References

1. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis. 2006;6(7):411–25.
2. King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet Lond Engl. 2005;365(9470):1561–9.
3. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet Lond Engl. 2014;383(9936):2253–64.
4. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop. 2000;77(1):41–51.
5. Friedman JF, Mital P, Kanzaria HK, Olds GR, Kurtis JD. Schistosomiasis and pregnancy. Trends Parasitol. 2007;23(4):159–64.
6. WHO | Neglected tropical diseases. WHO. World Health Organization; [cited 2020 Apr 25]. Available from: http://www.who.int/neglected_diseases/en/.
7. Fallon PG, Sturrock RF, Capron A, Niang M, Doenhoff MJ. Short report: diminished susceptibility to praziquantel in a senegal isolate of schistosoma mansoni. Am J Trop Med Hyg. 1995;53(1):61–2.
8. Fallon PG, Doenhoff MJ. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in schistosoma mansoni in mice is drug specific. Am J Trop Med Hyg. 1994;51(1):83–8.
9. De Clercq D, Vercruysse J, Picquet M, Shaw DJ, Diop M, Ly A, et al. The epidemiology of a recent focus of mixed c haematobium and schistosoma mansoni infections around the 'Lac de Guiers' in the Senegal River Basin, Senegal. Trop Med Int Health TM IH. 1999;4(8):544–50.
10. Wang W, Wang L, Liang Y-S. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. Parasitol Res. 2012;111(5):1871–7.
11. Borrmann S, Szlezák N, Faucher J-F, Matsiegui P-B, Neubauer R, Binder RK, et al. Artesunate and praziquantel for the treatment of schistosoma haematobium infections: a double-blind, randomized, Placebo-Controlled Study. J Infect Dis. 2001;184(10):1363–6.
12. Reinhard-Rupp J, Klohe K. Developing a comprehensive response for treatment of children under 6 years of age with schistosomiasis: research and development of a pediatric formulation of praziquantel. Infect Dis Poverty. 2017;6(1):122 [cited 2020 May 12]. Available from: https://doi.org/10.1186/s40249-017-0336-9.
13. Stete K, Krauth SJ, Coulibaly JT, Knopp S, Hattendorf J, Müller I, et al. Dynamics of Schistosoma haematobium egg output and associated infection parameters following treatment with praziquantel in school-aged children. Parasit Vectors. 2012;5:298.
14. Olliaro PL, Vaillant M, Diawara A, Coulibaly JT, Garba A, Keiser J, et al. Toward measuring schistosoma response to praziquantel treatment with appropriate descriptors of egg excretion. PLoS Negl Trop Dis. 2015;9(6) [cited 2020 Apr 26]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4473103/.
15. Montresor A. Cure rate is not a valid indicator for assessing drug efficacy and impact of preventive chemotherapy interventions against schistosomiasis and soil-transmitted helminthiasis. Trans R Soc Trop Med Hyg. 2011;105(7):361–3.

16. Dobson RJ, Sangster NC, Besier RB, Woodgate RG. Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test (FECRT). Vet Parasitol. 2009;161(1–2):162–7.

17. Engels D, Sinzinkayo E, De Vlas SJ, Gryseels B. Intraspecimen fecal egg count variation in Schistosoma mansoni infection. Am J Trop Med Hyg. 1997;57(5):571–7.

18. Mwinzi PNM, Kittur N, Ochola E, Cooper PJ, Campbell CHJ, King CH, et al. Additional evaluation of the point-of-contact circulating cathodic antigen assay for Schistosoma mansoni infection. Front Public Health. 2015;3 [cited 2020 Apr 20]. Available from: https://www.frontiersin.org/articles/10.3389/fpubh.2015.00048/full.

19. Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S. New diagnostic tools in schistosomiasis. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2015;21(6):529–42.

20. Stothard JR, Sousa-Figueiredo JC, Betson M, Bustinduy A, Reinhard-Rupp J. Schistosomiasis in African infants and preschool children: let them now be treated! Trends Parasitol. 2013;29(4):197–205.

21. Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, Downs JA. Examining the relationship between urogenital schistosomiasis and HIV infection. PLoS Negl Trop Dis. 2011;5(12) [cited 2020 Apr 19]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3232194/.

22. Hotez P. Female Genital Schistosomiasis (FGS): Sub-Saharan Africa's Secret Scourge of Girls and Women. [cited 2020 Apr 19]. Available from: http://blogs.plos.org/speakingofmedicine/?p=12631.

23. Ajanga A, Lwambo NJS, Blair L, Nyandindi U, Fenwick A, Brooker S. Schistosoma mansoni in pregnancy and associations with anaemia in northwest Tanzania. Trans R Soc Trop Med Hyg. 2006;100(1):59–63.

24. Friedman JF, Kanzaria HK, McGarvey ST. Human schistosomiasis and anemia: the relationship and potential mechanisms. Trends Parasitol. 2005;21(8):386–92.

25. Scientific Working Group on Schistosomiasis. Meeting (2005 : Geneva S, Diseases UBSP for R and T in T, Organization WH. Report of the Scientific Working Group meeting on Schistosomiasis, Geneva, 14–16 November, 2005. Scientific Working Group report on schistosomiasis, 14–16 November, 2005, Geneva, Switzerland. 2006 [cited 2020 Apr 26]. Available from: https://apps.who.int/iris/handle/10665/69482.

26. Team WHOSD and M for PD and VC. Report of the WHO informal consultation on the use of praziquantel during pregnancy/lactation and albendazole/mebendazole in children under 24 months: Geneva, 8-9 April 2002. 2003 [cited 2020 Apr 26]. Available from: https://apps.who.int/iris/handle/10665/68041.

27. Ndibazza J, Muhangi L, Akishule D, Kiggundu M, Ameke C, Oweka J, et al. Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. Clin Infect Dis Off Publ Infect Dis Soc Am. 2010;50(4):531–40.

28. Olveda RM, Acosta LP, Tallo V, Baltazar PI, Lesiguez JLS, Estanislao GG, et al. Efficacy and safety of praziquantel for the treatment of human schistosomiasis during pregnancy: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. 2016;16(2):199–208.

29. Brown DS, Sarfati C, Southgate VR, Ross GC, Knowles RJ. Observations on Schistosoma intercalatum in south-east Gabon. Z Parasitenkd Berl Ger. 1984; 70(2):243–53.

30. WHO | PCT databank. WHO. World Health Organization; [cited 2020 Apr 25]. Available from: http://www.who.int/neglected_diseases/preventive_chemotherapy/sch/en/.

31. World Bank Country and Lending Groups – World Bank Data Help Desk. [cited 2020 Apr 26]. Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups.

32. Mombo-Ngoma G, Honkpehedji J, Basra A, Mackanga JR, Zoleko RM, Zinsou J, et al. Urogenital schistosomiasis during pregnancy is associated with low birth weight delivery: analysis of a prospective cohort of pregnant women and their offspring in Gabon. Int J Parasitol. 2017;47(1):69–74.

33. Mombo-Ngoma G, Mackanga JR, González R, Ouedraogo S, Kakolwa MA, Manego RZ, et al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa: an observational multicountry study. BMJ Open. 2016;6(6) [cited 2020 Apr 25]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4932321/.

34. de Jonge N, De Caluwé P, Hilberath GW, Krijger FW, Polderman AM, Deelder AM. Circulating anodic antigen levels in serum before and after chemotherapy with praziquantel in schistosomiasis mansoni. Trans R Soc Trop Med Hyg. 1989;83(3):368–72.

35. van Dam GJ, Bogitsh BJ, van Zeyl RJ, Rotmans JP, Deelder AM. Schistosoma mansoni: in vitro and in vivo excretion of CAA and CCA by developing schistosomula and adult worms. J Parasitol. 1996;82(4):557–64.

36. Agnew A, Fulford AJ, De Jonge N, Krijger FW, Rodriguez-Chacon M, Gutsmann V, et al. The relationship between worm burden and levels of a circulating antigen (CAA) of five species of Schistosoma in mice. Parasitology. 1995;111(Pt 1):67–76.

37. Corstjens PLAM, DE Dood CJ, Kornelis D, Tjon Kon Fat EM, Wilson RA, Kariuki TM, et al. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. Parasitology. 2014;141(14):1841–55.

38. Corstjens PLAM, van Lieshout L, Zuiderwijk M, Kornelis D, Tanke HJ, Deelder AM, et al. Up-converting phosphor technology-based lateral flow assay for detection of Schistosoma circulating anodic antigen in serum. J Clin Microbiol. 2008;46(1):171–6.

39. van Dam GJ, de Dood CJ, Lewis M, Deelder AM, van Lieshout L, Tanke HJ, et al. A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. Exp Parasitol. 2013;135(2):274–82.

40. van Dam GJ, Xu J, Bergquist R, de Dood CJ, Utzinger J, Qin Z-Q, et al. An ultra-sensitive assay targeting the circulating anodic antigen for the diagnosis of Schistosoma japonicum in a low-endemic area, People's Republic of China. Acta Trop. 2015;141(Pt B):190–7.

41. Bergwerff AA, van Dam GJ, Rotmans JP, Deelder AM, Kamerling JP, Vliegenthart JF. The immunologically reactive part of immunopurified circulating anodic antigen from Schistosoma mansoni is a threonine-linked polysaccharide consisting of --> 6)-(beta-D-GlcpA-(1 --> 3))-beta-D-GalpNAc-(1 --> repeating units. J Biol Chem. 1994;269(50):31510–7.

42. Hoekstra PT, Schwarz N, Adegnika, AA, Rakotoandrianarivelo M, Corstjens P, Rakotoarivelo R, et al. Fast and reliable easy-to-use-diagnostics for eliminating bilharzia in young children and mothers: the freeBILy project. Acta Trop. 2020;211:105631. https://doi.org/10.1016/j.actatropica.2020.105631. Epub 2020 Jul 14.

43. Hürlimann E, Houngbedji CA, Yapi RB, Ndri PB, Silué KD, Soro G, et al. Health-related quality of life among school children with parasitic infections: findings from a national cross-sectional survey in Côte d'Ivoire. PLoS Negl Trop Dis. 2014;8(12) [cited 2020 May 19]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4256278/.

44. Dejon-Agobé JC, Zinsou JF, Honkpehedji YJ, Ateba-Ngoa U, Edoa J-R, Adegbite BR, et al. Schistosoma haematobium effects on Plasmodium falciparum infection modified by soil-transmitted helminths in school-age children living in rural areas of Gabon. Hsieh MH, editor. PLoS Negl Trop Dis. 2018;12(8):e0006663.

45. Ateba Ngoa U, Zinsou J, Kassa RF, Ngoune Feugap E, Honkpehedji Y, Massinga-Loembe M, et al. Assessment of the effect of Schistosoma haematobium co infection on malaria parasites and immune responses in rural populations in Gabon: study protocol. SpringerPlus. 2014;3(1):388.

46. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. BMC Public Health. 2017;17(1) [cited 2019 Feb 8]. Available from: http://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-017-4045-x.

47. Corstjens PLAM, de Dood CJ, Priest JW, Tanke HJ, Handali S, Peru and the CWG in. Feasibility of a lateral flow test for neurocysticercosis using novel up-converting nanomaterials and a lightweight strip analyzer. PLoS Negl Trop Dis. 2014;8(7):e2944.

48. Corstjens PLAM, Nyakundi RK, de Dood CJ, Kariuki TM, Ochola EA, Karanja DMS, et al. Improved sensitivity of the urine CAA lateral-flow assay for diagnosing active Schistosoma infections by using larger sample volumes. Parasit Vectors. 2015;8:241.

49. Corstjens PLAM, de Dood CJ, Knopp S, Clements MN, Ortu G, Umulisa I, et al. Circulating Anodic Antigen (CAA): a highly sensitive diagnostic biomarker to detect active schistosoma infections—improvement and use during SCORE. 2020. tpmd190819.

50. Organization WH. Basic laboratory methods in medical parasitology . World Health Organization; 1991. [cited 2020 May 13]. Available from: https://apps.who.int/iris/handle/10665/40793.

51. Obeng BB, Aryeetey YA, de Dood CJ, Amoah AS, Larbi IA, Deelder AM, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma

haematobium in urine samples from Ghana. Ann Trop Med Parasitol. 2008; 102(7):625–33.

52. de Dood CJ, Hoekstra PT, Mngara J, Kalluvya SE, van Dam GJ, Downs JA, et al. Refining diagnosis of schistosoma haematobium infections: antigen and antibody detection in urine. Front Immunol. 2018;9:2635.

53. WHO | Bench Aids for the diagnosis of intestinal parasites. WHO. [cited 2019 Feb 25]. Available from: http://www.who.int/intestinal_worms/resources/9789241544764/en/.

54. Colley DG, Binder S, Campbell C, King CH, Tchuem Tchuenté L-A, N'Goran EK, et al. A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of Schistosoma mansoni. Am J Trop Med Hyg. 2013;88(3):426–32.

55. Koga K, Kasuya S, Khamboonruang C, Sukhavat K, Ieda M, Takatsuka N, et al. A modified agar plate method for detection of Strongyloides stercoralis. Am J Trop Med Hyg. 1991;45(4):518–21.

56. WHO | Prevention and control of schistosomiasis and soil-transmitted helminthiasis: WHO Technical Report Series N° 912. WHO. [cited 2019 Feb 10]. Available from: http://www.who.int/intestinal_worms/resources/who_trs_912/en/.

57. Goldsmid JM. Studies on the laboratory diagnosis of human filariasis: preliminary communication. J Clin Pathol. 1970;23(7):632–5.

58. Joanny F, Löhr SJZ, Engleitner T, Lell B, Mordmüller B. Limit of blank and limit of detection of plasmodium falciparum thick blood smear microscopy in a routine setting in Central Africa. Malar J. 2014;13:234.

59. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377–81.

60. Adriko M, Standley CJ, Tinkitina B, Tukahebwa EM, Fenwick A, Fleming FM, et al. Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for Schistosoma mansoni in different transmission settings within Bugiri District, Uganda. Acta Trop. 2014;136:50–7.

61. Navaratnam AMD, Mutumba-Nakalembe MJ, Stothard JR, Kabatereine NB, Fenwick A, Sousa-Figueiredo JC. Notes on the use of urine-CCA dipsticks for detection of intestinal schistosomiasis in preschool children. Trans R Soc Trop Med Hyg. 2012;106(10):619–22.

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