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Diagnostic accuracy of point of care tests for *Schistosoma haematobium*: a systematic review

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Declaration

I, Erica Draxl (9500121E), am a student registered for a Master of Science in Epidemiology and Biostatistics in the academic year 2024. I hereby declare that the dissertation I am submitting for assessment contains no section copied in whole or in part from any other source unless explicitly identified in quotation marks with detailed, complete and accurate referencing. The contents of this dissertation are the original work of the author, Erica Draxl and was submitted to the Faculty of Health Sciences, School of Public Health in fulfillment of the requirements for the MSC (Epidemiology & Biostatistics) degree. No part of this work has been presented for any other degree or qualification.

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(Signature of candidate)

_____21st_____day of____October_____2024_____in___Johannesburg__

Dedication

To the community and patients of the complementary medicine practice Meygaarden Natural Health, who have allowed me the opportunity to spend time on completing this thesis.

Abstract

Background: Urogenital schistosomiasis (UGS) caused by *S. haematobium* is a serious public health problem in Sub-Saharan Africa, associated with increased HIV acquisition and transmission in co-endemic areas of HIV and UGS. Accurate and accessible point-of-care testing for screening and diagnosis is vital for control and prevention of UGS in Sub-Saharan Africa. Point-of-care (POC) rapid diagnostic tests that have the merit of high sensitivity for both high and low parasite burden have been developed. These tests need to be assessed for their applicability in poorly resourced UGS-endemic areas so that control programs can be initiated. This diagnostic test accuracy (DTA) review evaluates the sensitivity and specificity of different direct POC, rapid (RDT) and field-applicable diagnostic tests.

Objective: To assess the diagnostic accuracy of POC and rapid diagnostic tests for the direct diagnosis of urogenital schistosomiasis compared to laboratory-based reference tests in individuals from Africa, for improved screening and routine diagnosis in both endemic and non-endemic settings.

Methods: Four databases were systematically searched for DTA studies on *S. haematobium* exposed patients in endemic and non-endemic settings from 2000-2022. Studies that compare the sensitivity and specificity of any direct POC or RDT for *S. haematobium* to a reference test were included. Using the variability of test thresholds, a bivariate or HSROC model was used in STATA 17 and Revman 5.3 software to calculate the summary ROC model for each eligible test. Heterogeneity tests and subgroup analyses for different reference standard comparators, age-groups and prevalence settings were conducted. Results were presented as percentages with a 95% confidence interval.

Results: Thirty-three studies were evaluated, in which ten different POC and rapid tests were eligible, of which seven were included in a meta- analysis. Two field based molecular tests; loop mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) (sensitivity=99.9%, specificity=76.2% and sensitivity=94,1%, specificity=96.6% respectively) showed the most promise compared to a laboratory test. The immunochromatographic rapid diagnostic test (ICT-RDT) and *S. mansoni* cercarial transformation fluid RDT (SmCTF-RDT) serological tests are easy-to-use POC cassettes and have high sensitivity (96% and 98.4% respectively) but both require a centrifuge to obtain serum samples. Other serological tests: dipstick dye immunoassay (DDIA) and indirect haemagglutination assay (IHA) had lower sensitivities. The

POC circulating cathodic antigen test (POC-CCA) (sensitivity=47%, specificity=71%) is not suitable for *S. haematobium* in prevalence settings of less than 50%. The up-converting phosphor lateral flow circulating anodic antigen test (UCP-LFCAA) (sensitivity=73%, specificity=72%), although laboratory intensive shows promise in endemic areas.

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- Contributions were made with thanks by Daniel Elakpa in the data extraction process as a second reviewer.

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LIST OF ABBREVIATIONS/NOMENCLATURE

AIDS	Acquired Immunodeficiency Syndrome
ASSURED	Affordable, Sensitive, Specific, User-friendly, Robust & Rapid, Equipment free, Deliverable
CAA	Circulating Anodic Antigen
CCA	Circulating Cathodic Antigen
CI	Confidence Interval
CTF	Cercarial Transformation Fluid
DALY	Disability Adjusted Life Years
DDIA	Dipstick Dye Immunoassay
DNA	Deoxyribonucleic acid
DOR	Diagnostic Odds Ratio
DTA	Diagnostic Test Accuracy
ELISA	Enzyme-linked Immunosorbent Assay
FGS	Female Genital Schistosomiasis
FN	False Negative
FP	False Positive
HIV	Human Immunodeficiency Virus
ICT-RDT	Immunochromatographic Rapid Diagnostic Test
IHA	Indirect Haemagglutination Assay
LAMP	Loop mediated isothermal amplification
LAMP-PURE	Loop mediated isothermal amplification- procedure for ultra rapid DNA extraction
LCA	Latent Class Analysis
LF	Lateral Flow
MDA	Mass Drug Administration
NM1	Newton microscope
NTD	Neglected Tropical Diseases
PCR	Polymerase chain reaction
POC	Point of Care
POC-CCA	Point-of-Care Circulating Cathodic Antigen
PRISMA	Preferred Reporting Items for a Systematic Review and Meta-analysis
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RDT	Rapid Diagnostic Test
RDT-Sh	Rapid Diagnostic Test-*S. haematobium*

RNA	Ribonucleic acid
ROC	Receiver Operating Characteristic
RPA	Recombinase Polymerase Amplification
SDTM	Cochrane Screening and Diagnostic Test Methods Group
Sh-Dra1	*S. haematobium* 121 base pair repeated sequence
SmCTF-RDT	*S. mansoni* Cercarial Transformation Fluid Rapid Diagnostic Test
SROC	Summary Receiver Operating Characteristic
TMB	Tetramethylbenzidine
TN	True Negative
TP	True Positive
UCP	Up-converting Phosphor
UCP LF-CAA	Up-converting Phosphor Lateral Flow Circulating Anodic Antigen Test
UK	United Kingdom
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background

Schistosomiasis, or bilharzia, is a water-borne neglected tropical disease caused by the parasitic blood flukes of the genus *Schistosoma* and is a serious public health problem in sub-Saharan Africa. Schistosomiasis affects children and young adults, living in the blood vessels for years by evading the immune system while continually excreting eggs(1). More than 240 million people worldwide are affected, 90% of whom are in Africa. The debilitating effects of schistosomiasis and the burden of disease due to malnutrition, internal hemorrhage and end organ failure contributes to 70 million disability adjusted life years (DALY's) lost annually, similar to HIV and higher than malaria(2).

Efforts towards the elimination of schistosomiasis has been successful in Morocco, Japan and Tunisia, while Egypt, Brazil and China are making progress towards the full elimination of the disease(3). Sub-Saharan Africa unfortunately bears the brunt of the highest incidence of schistosomiasis, where persistent transmission continues, especially in poor rural communities.

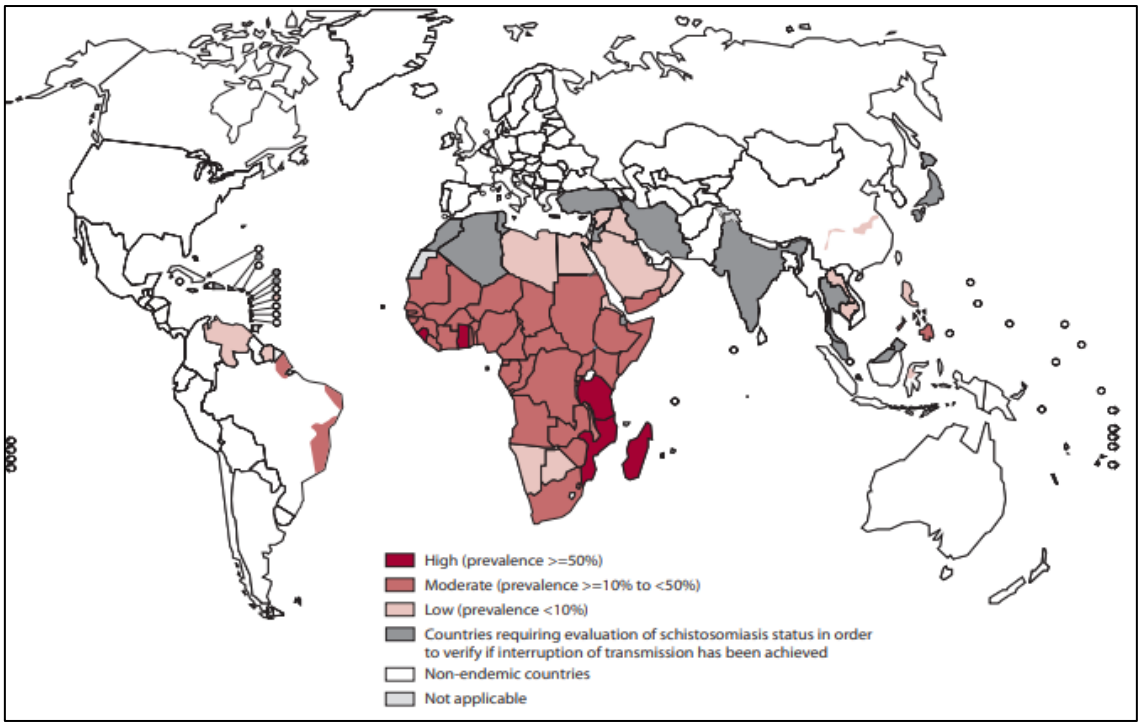


Figure 1: Geographic distribution worldwide and prevalence of schistosomiasis, 2010

The need for improved diagnostic tools has been increasingly recognized by the World Health Organization (WHO) and schistosomiasis research communities as an imperative first step to

improve control strategies, and for improved geographic mapping and prevalence in endemic areas. Fast, reliable and near-patients diagnostic tests are an integral part of sustained control programs and decision-making for schistosomiasis elimination programs.

1.1.1 Neglected Tropical Diseases

Schistosomiasis is one of 20 conditions prioritized by the WHO as a neglected tropical disease (NTD), due to its devastating effect on impoverished communities(4). NTD's are considered preventable and treatable, affecting the poorest and most vulnerable people. Lack of basic hygiene, sanitation and access to healthcare facilities often means that infection is unavoidable in endemic areas, where washing and swimming in infected water is a daily risk factor for multiple infections, and even re-infection after treatment. When left untreated, schistosomiasis can progress to severe chronic disease because of the granulomatous inflammatory reaction in organs and tissues towards the schistosome eggs(5). Schistosomiasis can be cured without progressing to complications if early-stage diagnosis is made and prompt treatment is administered (5).

1.1.2 Clinical features and public health implications

Sub-Saharan Africa is affected by two types of schistosomiases; urogenital schistosomiasis, caused by *Schistosoma haematobium* and intestinal schistosomiasis, caused by *Schistosoma mansoni* (5). Both are acquired by exposure to infected water harboring specific snail species that serve as intermediate hosts for *Schistosoma* species (3). *Schistosoma* parasites live an average of 3-10 years in genitourinary or mesenteric veins but can endure for up to 40 years(6) resulting in a lifetime of chronic ill health.

Intestinal schistosomiasis results in lesions in the intestinal mucosa and liver which can result in bleeding, malnutrition, and advanced disease manifestations and even death due to fibrosis in the portal veins of the liver. Urogenital schistosomiasis results in pathology from damage to the bladder, kidneys, vaginal mucosa and genital organs resulting in symptoms such as painful urination and hematuria and can advance to bladder cancer or obstructive uropathy(5).

Schistosomiasis of the lower reproductive tract caused by *S. haematobium* is called female genital schistosomiasis (FGS) or male genital schistosomiasis and leads to infertility and an increased risk of acquiring sexually transmitted diseases including HIV(7). Numerous studies in sub-Saharan Africa have ascertained that FGS is associated with a 2.9 to 4-times higher risk of HIV acquisition(7).

1.1.3 Epidemiology and distribution of schistosomiasis in South Africa

In South Africa, an estimated 4.5 million new infections occur annually, and up to 27.5 million people are estimated to be exposed to water infected with *Schistosoma*(8). Schistosomiasis infection is predominantly the urogenital type in South Africa, where prevalence data from national laboratory testing in 2011-2018 found 0.2% *S. mansoni* compared to 99.8% *S. haematobium*(7). *S. haematobium* predominance occurs endemically in KwaZulu Natal, Mpumalanga and Eastern Cape(3), while mixed *S. mansoni /S. haematobium* occurs in Limpopo province(9). Regional studies and surveys carried out in South Africa between 2001 and 2017 demonstrated a high prevalence of urogenital schistosomiasis infection ranging from 24 to 98% in school-aged children aged 10-14 years in these regions (10), and around 37,5% in the general population(11). A high prevalence of cases is diagnosed in all major metropolitan areas due to migrants and travellers from endemic areas(9). Figure 2 shows prevalence estimates from data collated from National Health Laboratory (NHL) during the period 2011-2018(9).

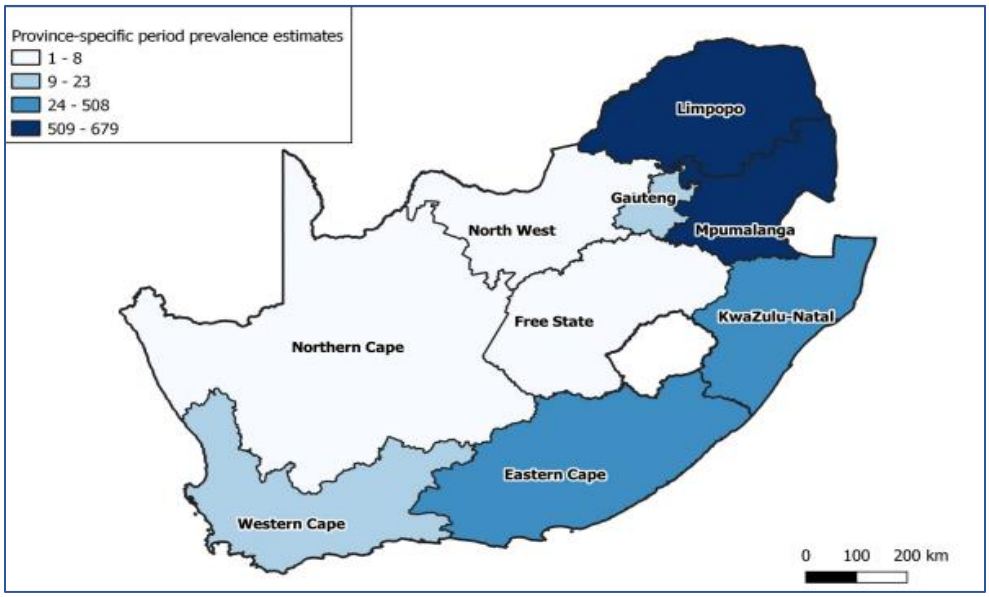


Figure 2: Province specific period prevalence estimates of microscopically confirmed schistosomiasis. South Africa 2011-2018

Children aged 9-15 years have the highest prevalence of schistosomiasis in South Africa (52% of cases), due to recreational swimming, or washing in infected waterbodies(9). A prevalence study of FGS in girls and young women in KwaZulu-Natal by Livingston et al (2021), found a baseline prevalence of urinary schistosomiasis and FGS in 34% of schoolgirls aged 10-12 years, and 25% in secondary school learners aged 16-19 years.

1.1.4 Target condition being diagnosed

This review will focus on the diagnosis of urogenital schistosomiasis caused by the parasitic blood fluke *S. haematobium.* Urogenital schistosomiasis is the most prevalent type of schistosomiasis in South Africa, and its chronic genital sequalae are specifically associated with higher incidence of HIV/AIDS representing an important public health risk. The rationale to focus on *S. haematobium* only, is for the improvement of urgently needed diagnosis and screening for urogenital schistosomiasis which has thus far been neglected, thereby improving successful implementation of control programs in sub-Saharan Africa and the Southern African region where urogenital schistosomiasis is more prevalent.

1.1.5 Clinical Pathway

At present in South Africa, urogenital schistosomiasis is diagnosed and treated in regional primary healthcare clinics, where all patients who present with haematuria/ microhaematuria by urine dipstick should be tested by microscopic examination of a urine specimen(12). The management of schistosomiasis is limited to case-based treatment for those who seek medical care for blood in their urine, however there are no awareness programs or systematic testing for hematuria in South African clinics(13). Due to poor health seeking behaviour for initial symptoms, as well as poor awareness of chronic sequalae by the public and health professionals, many cases go untreated and progress to the chronic stage of the disease(14).

1.1.6 Current diagnostic methods for *S. haematobium*

Parasitological methods using microscopy of urine for egg detection are widely used for the diagnosis of urogenital schistosomiasis. A single filtration of 10ml of urine through a 20µm Nucleopore ™ filter is a standard field- based technique, although centrifuging and sedimentation is also used to concentrate the urine. The filter is stained with Lugol's iodine and examined using a light microscope 10X - 40X magnification to detect schistosome eggs. This method requires equipment and technical expertise and can be time consuming making it unsuitable for mass field screening. Surveillance using microscopy of filtered urine to detect eggs leads to a strong bias toward false negatives which is detrimental to epidemiological prevalence studies(15). Urine microscopy has a low sensitivity in areas of low schistosomiasis prevalence (16) and long-standing infections such as FGS(17). The reason is that the sensitivity of the test differs due to discontinuous emission of eggs, so that the detection of eggs in urine varies daily.

Although urine microscopy has low sensitivity when few eggs are being released into urine, its specificity is high (approximately 100%) as there are seldom false positives; an egg detected construes a positive result. However, screening using urine microscopy was found to underestimate the infection prevalence in pre-school and school-aged children from an endemic area in rural Zimbabwe(18). They showed that infection prevalence was 37,4% using microscopy and 71.5% using serology, and 86.3% using urine dipsticks for haematuria(18). In women with FGS only 23% were found to have eggs in a urine sample tested using microscopy (17). The low sensitivity of microscopy is especially problematic when testing after mass drug administration (MDA) programs, due to lower infection intensity.

Although microscopy of urine is the recommended reference test considered a 'gold standard' for diagnosis, its sensitivity is only reliable where high infection intensity occurs endemically such as in 'hotspots' where the infection rate is >50%(19). This discrepancy indicates the need for more sensitive tests to replace the conventional parasitological method of microscopy that is currently recommended for prevalence evaluation. A test that is sensitive for all stages of the infection including chronic, as well as in low prevalence areas, is needed for accurate control programs.

1.1.7 Control strategies in South Africa

Despite the high prevalence of schistosomiasis in five provinces, there are no policies in place in South Africa to control the transmission. Control strategies endorsed by the WHO since 1985(20) are focused on annual mass drug administration to 75% of school-aged children in endemic areas with a microscopically determined infection prevalence of >10%(19) However, this strategy has failed in South Africa due to a low coverage, regulations on generic medication, and challenges in implementation(14). Currently, Praziquantel is the only drug recommended for the regular treatment of schistosomiasis in South Africa at a dose of one annual treatment of 40mg/kg (4).

A recent review in 2022 by Nemungadi *et al* has highlighted the urgent need for implementation of a formal and funded control program in South Africa. There is a high burden of FGS in South Africa due to untreated urogenital schistosomiasis, which is estimated to increase by 30% in the next decade unless infected girls are treated(13). FGS, due to its chronicity, is difficult to diagnose, and can present with a range of symptoms, from pelvic discomfort to vaginitis-type symptoms(21). At present FGS has no rapid method of testing, requiring coloscopy (internal examination of cervix and vaginal mucosa) by trained medical staff, or expensive laboratory testing(22). The success of future schistosomiasis control programs is intrinsically associated with improved diagnostic tests for better compliance, and epidemiological surveillance.

1.1.8 Point-of-care diagnostic testing

The ideal test for resource limited settings would be a point-of-care test, that is easy to use, highly sensitive and specific for all *Schistosoma* species, and uses samples that do not require invasive collection procedures (e.g., urine or saliva instead of blood). Drain *et al* (2014) defines point of care (POC) testing as "a test that is conducted rapidly at or near the site of clinical care, with the aim to facilitate timely and cost-effective decision making"(23).

1.2 Problem statement

Urogenital schistosomiasis remains a public health burden in sub–Saharan Africa with significant morbidity particularly in impoverished communities. *S. haematobium* is responsible for egg-induced pathological lesions of the urogenital tract in both men and women, with implications for transmission of co-morbidities such as HIV. Southern Africa is considered a geographical hotspot for urogenital schistosomiasis. An effective approach for policymakers and clinicians is the development of more sensitive diagnostic tool for improved screening, control, and elimination of schistosomiasis. Due to the minimally symptomatic nature of this disease, and poor awareness of the consequences of untreated schistosomiasis, high transmission rates can continue if infected individuals urinate near or in water bodies.

The diagnosis of both acute and chronic stages of urogenital schistosomiasis remains a challenge to accurately determine the epidemiology and staging of the disease(24). The ideal method to ensure rapid diagnosis of urogenital disease has not been fully assessed. To date, there is no systematic review published to synthesize the various point-of-care or rapid tests available specifically for the direct diagnosis of *S. haematobium* in various settings of prevalence.

1.3 Justification

This is the first diagnostic test accuracy (DTA) review that assesses the direct diagnosis of the *S. haematobium* parasite that causes urogenital schistosomiasis, using field applicable tests for low resource settings. There is a diagnostic gap for this disease especially in low prevalence settings and for chronic disease such as female and male genital schistosomiasis. Although previous reviews include diagnostic tests for both urogenital and intestinal schistosomiasis, this review will guide clinicians in regions where *S. haematobium* is the predominant species, specifically for the diagnostic accuracy of POC tests as alternatives or concurrent to microscopy of filtered urine for egg detection.

The rationale for policymakers is to compare these tests' performance for implementing control programs, based on the WHO recommendations: 1) screening and prevalence 2) community mapping in low and high prevalence settings 3) test and treat strategies 4) monitoring of interruption of transmission for post MDA and low intensity infections.

For real field applications demanded by resource limited settings, the ASSURED test criteria (Affordable, Sensitive, Specific, User-friendly, Robust & Rapid, Equipment free, Deliverable) for diagnostic tests was developed by the WHO (25), and currently there is no such test available for *S. haematobium.* In contrast, intestinal schistosomiasis has an ASSURED test available which is rapid, POC and commercially available for more than a decade to test for *S. mansoni*, that is, the circulating cathodic antigen test (POC-CCA) (26).

Using POC tests for a test-and- treat protocol for urogenital schistosomiasis could become part of the diagnostic work-up in clinics in endemic areas and will therefore enable diagnosis of subclinical and chronic infections that do not display haematuria.

1.4 Research Question

What is the diagnostic performance of rapid and point-of-care diagnostic tests for the direct diagnosis of urogenital schistosomiasis compared to laboratory-based reference tests in individuals from Africa?

1.5 Aim

The aim of the study is to assess the diagnostic performance of rapid and point-of-care diagnostic tests for the direct diagnosis of urogenital schistosomiasis compared to laboratory-based reference tests in individuals from Africa.

1.6 Objectives

1. To obtain summary estimates of the diagnostic accuracy of eligible direct point-of-care or rapid tests for the diagnosis of active urogenital schistosomiasis using sensitivity and specificity values, where an appropriate laboratory test is used as the reference standard.
2. To compare the accuracy of eligible direct point-of-care or rapid tests for the diagnosis of active urogenital schistosomiasis.
3. To investigate potential sources of heterogeneity in the diagnostic accuracy of each index test assessed, focusing specifically on the implications of age, type of reference standard used, and prevalence or endemicity setting on the heterogeneity observed.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter reviews the relevant literature on diagnostic accuracy and performance of rapid or POC tests for *S. haematobium*. It presents the details on how diagnostic accuracy studies are performed, details about the reference test/s used as a comparator, other diagnostic test accuracy reviews for schistosomiasis testing, and their differences with this review. The literature around the current available index tests for *S. haematobium* are reviewed and presented in a table.

2.2 Diagnostic test accuracy reviews: background and considerations for schistosomiasis

The aim of a DTA review is to address a need for health policy makers to have access to information about the diagnostic accuracy of possible tests to use in healthcare settings. The aim is to minimize bias and improve reliability by synthesizing results(27). This review focuses on rapid tests for *S. haematobium* only, while all other DTA studies in the literature focus on both types of schistosomiases, including tests for both *S. haematobium* and *S. mansoni*. While some tests can distinguish between both species, or have equal ability to diagnose either species, most tests perform differently for each species. Therefore, comparisons and accuracy studies for index tests should ideally be performed separately for each species of *Schistosoma*.

2.2.1 Diagnostic accuracy measures

Essentially a diagnostic test should provide an accurate diagnosis, and a repeat test after some time determines if the treatment given has eliminated the infection. Diagnostic test accuracy refers to the test's ability to detect the presence of disease, that is its discriminative ability(27). In this review, measures of sensitivity and specificity were calculated to determine the diagnostic accuracy of a test. Test sensitivity is defined as "the probability that the index test result will be positive in a diseased case". Test specificity is defined as "the probability that the index test result will be negative in a non-diseased case". In order to calculate sensitivity and specificity, measures of the index test should be cross-referenced with a gold standard test that has a known high accuracy. True positives (TN), false positives (FP), true negatives (TN) and false negatives (FN) are calculated for the index test and used to populate a 2x2 contingency table from which sensitivity as a/(a+c) and specificity as d/(b+d) are calculated (see Table 1).

Table 1: 2x2 cross classification of test results and disease status(27)

Index test outcome	**Disease status (reference standard result)**		
	Diseased (D+)	Non-diseased (D-)	Total
Index test positive (T+)	True positives (a)	False positives (b)	Test positives (a+b)
Index test negative(T-)	False negatives (c)	True negatives (d)	Test negatives (c+d)
	Disease positives (a+c)	Disease negatives (b+d)	N (a+b+c+d)

2.2.2 Challenges with microscopy as the standard reference

For urogenital schistosomiasis, the conventional reference test in many test performance studies is microscopy of urine for egg detection. Microscopy can be considered an imperfect reference test, due to its notoriously low sensitivity. A new test with superior sensitivity would be penalised in a test performance study by being assigned a low specificity(19). As a result, many studies use repeated microscopy measurements to improve the sensitivity of microscopy for egg detection and to accommodate for variations in egg excretion. In low prevalence settings some studies use a composite reference standard where two or more tests are used together, for example both microscopy and PCR of urine. In the absence of an improved reference standard, some studies use a statistical model called Latent Class Analysis (LCA) where multiple test results are combined in order to construct a reference standard(28).

2.2.3 Challenges with results for varying age-groups, and prevalence settings

Age group: The highest intensity of ovum excretion by schistosomes occurs in children ages 10 to 14 years and declines to low levels in adulthood(20). Pre-school children (<7 years) can be considered as new infections and have the possibility of giving accurate results for all types of tests including serological tests. This means that in this age group a positive antibody result should infer an active infection. Older school aged children (8-15 years) and adults (>16years) cannot be considered having an active infection from a serological result, as they could have a previous infection that was treated, especially after MDA in an area. The optimal egg excreting age group (8-15 years) can cause bias for index tests and reference tests that rely on eggs in urine when compared to an adult age group with minimal egg excretion, or a chronic infection.

Prevalence setting: Index tests may perform better for samples that contain a high level of schistosome antigen in endemic/high intensity setting and moderate intensity settings. Low intensity (including post-elimination/low endemic) setting are a challenge for many tests and cannot be compared to samples from a high intensity setting. Areas where schistosomiasis does

not exist but where testing is done for non-immune travellers coming from an endemic area are ideal for serological tests, but where testing is done on sub-Saharan migrants who harbour chronic schistosomiasis, these same tests are not valid for determining active infection.

2.2.4 Indirect and Direct tests

Tests for schistosomiasis can be direct, or indirect. Direct methods detect evidence of the schistosome itself, such as microscope examination of urine for schistosome eggs (parasitological tests), or tests to detect schistosome antigens from eggs or worm proteins (immunological tests), and detection of schistosome- specific DNA (molecular tests). Indirect methods are those that use questionnaires, urine reagent strips and ultrasound scans as proxy tests, and are more suited to screening in large populations, not individual diagnosis(29). For instance, the WHO recognizes haematuria presence using a urine dipstick as an additional test to microscopy for identifying areas of high prevalence(30), and is considered by Grolimund *et al* (2022) to be a good proxy for prevalence estimates of *S. haematobium* infection(31). However, indirect tests such as reagent urine strips cannot replace a direct diagnostic test to determine transmission interruption and are more suited to prevalence screening(29). In this review we will exclude indirect tests such as questionnaires and urine reagents strips, and only include direct tests that have a rapid or POC platform.

2.3 Diagnostic tests available for urogenital schistosomiasis

The information about various testing methods for urogenital schistosomiasis are introduced in this section by reviewing the information contained in other DTA systematic reviews. Tests are divided into laboratory tests (requiring extensive equipment and expertise), or field-based rapid and POC tests (requiring minimal equipment and easy to administer). Indirect tests for urogenital schistosomiasis screening are urine dipsticks and have been extensively studied. Direct tests are further divided into antigen tests, antibody tests (serology), and molecular (DNA based) tests and are included in this review.

2.3.1 Laboratory tests

Medical laboratories with high-tech equipment and proper resources are not feasible for many areas of sub-Saharan Africa where *Schistosoma* is endemic. However, laboratory tests have the high diagnostic performance needed for effective control programs, especially when infection prevalence is low after mass drug administration, and to determine interruption of transmission. Low prevalence settings (<10% in school aged children) are a challenge for many tests that rely on

a high level of eggs or antigens to be accurate(32). In 2014, Gomes *et al* published a systematic review on all laboratorial methods for schistosomiasis diagnosis, specifically focusing on tests that are validated by large evaluations in field(33). The results show that there are indeed very effective and sensitive laboratory tests for *S. haematobium*, including Western Blot and polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA)(33) with high sensitivities of 94,8%, 92% and 94,7% respectively(33) (34).

2.3.1.1 Serology using ELISA

ELISA is considered the most diagnostically accurate serological assay and detects antibodies to various *Schistosoma* antigens using a laboratory intensive method, and its use in the field is limited as it requires cold-chain logistics, laboratory equipment and overnight incubation. Hinz *et al* (2017) published a narrative review on the evaluation of serological assays that detect antibodies against all *Schistosoma* species in humans(35). For *S. haematobium* 26 antigenic targets are identified and antibody detection for egg and adult worm antigens using ELISA had very high sensitivities of between 87-100%. Of the many antigens, the most commonly used for screening is microsomal antigen *S. haematobium* (HAMA) and soluble worm antigen (SEA). A commercially available field-based ELISA-SEA kit is available, using finger-prick blood, with a sensitivity of 89% and specificity of 70% in an endemic population(36). Drawbacks of SEA-ELISA however, is its lack of specificity due to cross reactions with intestinal helminths, as well as chronicity of the IgG antibody to SEA, and therefore this tests is not used widely for screening of urinary schistosomiasis.

2.3.1.2 Molecular tests (PCR) using DNA or RNA detection

PCR has the diagnostic potential of detecting *S. haematobium* in serum during the acute phase and chronic phase of infection and is highly sensitive at 95-100% from 5 weeks after infection with little day to day variation before and after treatment(37). The real-time PCR requires easily obtained samples to detect DNA, either urine sedimentation on filter paper or fingerpick blood drop. DNA extraction from the sample, and DNA amplification steps require high-tech and expensive equipment, which is not portable and only suited to properly resourced laboratories. PCR approaches are considered the most reliable test for monitoring responses to treatment. Melchers *et al* (2014) found that PCR detected 69,3% infection prevalence after Praziquantel chemotherapy versus 22,8% by microscopy for *S. haematobium*(37).

Many schistosomiasis-endemic communities are poorly resourced and undeveloped, these in-house and often expensive laboratory tests may not be feasible for screening and elimination studies.

2.3.2 Field-based rapid and POC tests

POC tests are defined as those that can be conducted at or near the clinical site(23), and rapid tests are laboratory tests that use field-based kits for poor-resourced laboratories, and where the results are available within 2 hours. Due to the resource limitations of laboratory-based tests, adaptions of laboratory tests for field-applicability are an important research focus(38). Different antibody tests have been adapted into rapid lateral flow cassettes for POC use(39) (40). Innovative methods to extract and amplify DNA are also becoming available for field-based feasible alternatives to PCR(41)(42). Field versions of laboratory tests can be effective rapid tools, and use reagents that are not heat sensitive, utilising portable table-top mini centrifuging devices, and still ensure results are available within 2 hours.

Despite new POC and field applicable innovations in diagnostic testing, the updated 2022 WHO guideline on control and elimination of human schistosomiasis(19) found that there is still insufficient evidence to support using new diagnostic methods beyond the conventional urine microscopy.

2.3.2.1 Indirect tests: Urine reagent strips

In lieu of effective rapid direct tests, urine reagent strips are widely used as a sensitive screening test for large populations in endemic areas (30). The strips are cheap, quick and easy-to-use and have no technical requirements. Overall urine dipstick performance showed 81% sensitivity and 89% specificity for egg positive urine in a meta-analysis by King *et al* (2013). A urine dipstick is still an effective proxy test even in low prevalence areas and post treatment areas, where sensitivity only decreased to 79% and 81% respectively(43). Stothard *et al* (2014) revealed that urine dipsticks are sensitive as a proxy for egg detection for school-aged children in endemic areas(44), and should be interpreted within a setting where risk for schistosomiasis is evident. Urine dipsticks, although effective for prevalence screening, are indirect tests and unfortunately their results must be verified by a direct test to detect *Schistosoma* eggs, antigens, antibodies or DNA. Therefore, urine dipsticks are not included in this review for analysis.

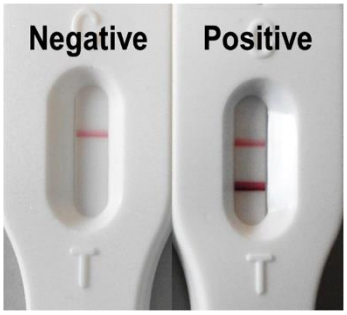
2.3.2.2 Antigen-Antibody tests (Serology):

Antibody tests using serum: Serology assays elucidate whether there is an antibody response to various schistosome antigens. Field-based serological tests detect antibodies against soluble worm antigen or egg antigen by indirect haemagglutination(45) or immunochromatography(46,47). Examples of field based rapid antibody tests are the IHA (indirect haemagglutination assay), the DDIA (dipstick dye immunoassay), the SmCTF-RDT (*S.mansoni*-cercarial transformation fluid-RDT) and the ICT-RDT (immunochromatographic rapid test)(see Table 2). All four tests are rapid, but use serum samples, which needs a centrifuge in a laboratory setting to spin blood samples. These tests are not species specific, and a positive result does not determine if the species is *S. mansoni*, or *S haematobium.*(35) However, they are effective in control programs where the effective treatment strategy is the same for both species.

The limitation with serological tests is that they do not diagnose whether the person tested is currently infected, or if antibodies from a past infection are diagnosed. Therefore, these tests are more suitable for first time infections in young children. They are also usually the test of choice for returning travellers who have never been exposed previously to schistosomiasis(32).

Table 2: Available serological field based rapid and POC antibody tests.

Index Test	Details of the index test:	Image of test
IHA: Indirect haemagglutination assay	**Type of test**: Rapid, in laboratory **Sample**: Serum **Method of action**: agglutination observed with naked eye for positive sera. **Field applicability**: simple test requiring only basic laboratory equipment. **Time to result**: 1 hour **Species specific**: No. Cannot distinguish between *S. haematobium* and *S. mansoni*. **Manufacturer:** Fumouze Laboratories	 Hemagglutination reaction observed for IHA test
ICT-RDT: Immunochromatographic test-RDT	**Type of test**: Rapid, lateral flow cassette **Sample:** Serum **Method of action**: IgG and IgM antibodies are detected in serum towards *Schistosoma spp* antigen. 30ml of serum mixed with 3 drops of eluent on a sample pad in lateral flow cassette, allowing control/test band to colour. **Field applicability**: simple to use, only a table-top centrifuge required to attain serum. **Time to result:** 20-30 minutes **Species specific**: No. Cannot distinguish between *S. haematobium* and *S. mansoni*. **Manufacturer**: LDBIO Diagnostics	 ICT-RDT lateral flow test

Index Test	Details of the index test:	Image of test
Sm-CTF RDT: *S.mansoni*-cercarial transformation fluid-RDT	**Type of test**: Rapid, lateral flow cassette **Sample**: Serum **Method of action**: Freeze-dried *S.mansoni* cercarial transformation fluid applied to the test band on a lateral flow cassette to elicit an antibody response to both *S. mansoni* and *S. haematobium* in serum. **Time to result:** 15 to 20 minutes **Field Applicability**: Table- top centrifuge required to attain serum. **Species specific:** No. Cannot distinguish between *S. haematobium* and *S. mansoni.* **Manufacturer**: Vision Biolab, South Africa	 Sm-CTF-RDT lateral flow test cassette
DDIA: Dipstick Dye Immunoassay	**Type of test**: Rapid, simple laboratory process **Sample**: Serum **Method of action**: The test consists of a dipstick and a blue colloidal dye labelled SEA solution that is added to a PVC well with a 20 µl serum specimen. **Time to result**: 20 minutes **Field Applicability**: Table- top centrifuge required to attain serum. **Species specific:** No. Cannot distinguish between *S. haematobium* and *S. mansoni.*	
RDT-Sh: Rapid Diagnostic Test for *S. haematobium*	**Type of test**: Rapid, field-based device **Sample**: Urine **Method of action**: The test consists a coupling device and a filter. Urine is mixed with anti-IgG conjugated to horseradish peroxidase and is taken up into the syringe and pushed through. Any eggs are trapped in the filter are stained blue. **Time to result:** 20 minutes **Field Applicability**: Yes, simple and cheap **Species specific:** Yes, Tests for *S. haematobium* only though egg detection.	 Syringe attached to coupling device and filter

Antigen tests using urine: The detection of adult worm antigens released in urine may be a more useful method of diagnosis in endemic areas, as these tests are able to detect an active worm burden, inferring an active infection. Antigen tests detect antigens released into the blood by the adult worm, usually two types: circulating cathodic antigen (CCA) and circulating anodic antigen (CAA). The easy-to-use POC-CCA cassette which has been available for more than a decade to effectively diagnose *S. mansoni*, does not perform well for *S. haematobium.* Danso-Appiah *et al* (2015) and Ochodo *et al* (2015) published systematic reviews on the point-of-care circulating cathodic antigen (POC-CCA) test for the detection of all schistosome infections(26) (48). Both reviews found poor sensitivity and specificity of the CCA test for *S. haematobium* compared to the

reference test of filtered urine microscopy. Ochodo *et al* revealed a sensitivity of 39% and specificity of 78% for the POC-CCA test for *S. haematobium*. The systematic review by Danso-Appiah revealed that the POC-CCA cassette is a good proxy test to replace microscopy for *S. mansoni*, but not for *S. haematobium*. It was found by Rubaba *et al* (2018) that the POC-CCA test has poor accuracy for *S. haematobium* in areas where a prevalence is <50%(49).

Other rapid antigen tests include the UCP-LFCAA using circulating anodic antigen (CAA) released by the adult worm(50). CAA can be detected in serum and urine within 3-4 weeks post-infection and is valuable for the determination of an active infection, as well as therapeutic response after treatment(51). This test has proven to be an ultra- sensitive quantitative technique that detects all *Schistosoma* species. This is a lateral flow (LF) based test that applies an ultrasensitive fluorescent label called upconverting phosphor (UCP) for the detection of the *Schistosoma* CAA using a portable strip reader. The test in its current form requires centrifuging and concentration techniques which still require laboratory equipment, so field applicability is limited. However, this test is considered a near-patient, field applicable test using portable laboratory equipment.

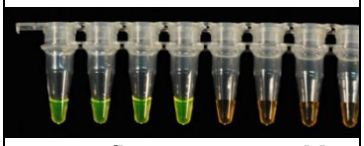
Table 3: Available field based rapid and POC antigen tests

Index Test	Details of the index test:	Image of test
POC-CCA: Point of Care- Circulating Cathodic Antigen	**Type of test**: POC, lateral flow cassette **Sample**: Urine **Method of action**: The POC-CCA is a urine based lateral flow cassette that detects CCA from the adult Schistosoma worm in urine. **Time to result:** 20 minutes. **Field Applicability**: Yes, 1 drop of urine and buffer needed. **Species specific:** No. Cannot distinguish between species but known to have poor accuracy for *S. haematobium.*	 POC-CCA lateral flow test for urine and buffer
UCP-LF CAA: Up-converting Phosphor-Lateral Flow Circulating Anodic Antigen (specifically UCAA2000)	**Type of test**: Rapid, near patient but laboratory-based **Sample**: Urine for the UCAA2000, or serum for the SCAA500 **Method of action**: The CAA antigen is detected using luminescent quantitative up-converting phosphor (UCP) and is a lateral flow (LF) based assay. **Time to result:** 20 minutes **Field Applicability**: Table- top centrifuge required to attain serum. Portable UCP scanner needed to read the results. **Species specific:** No, detects all *Schistosoma* species, and does not distinguish species.	 UCP-LF CAA with lateral flow strips and portable scanner

2.3.2.3 Field-based DNA-based molecular tests

New advances in molecular DNA-based field applicable tests are loop mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), both of which are approaching status as a rapid diagnostic test (RDT). DNA extraction for LAMP is possible using a field- based LAMP-PURE kit and amplification are steps done within 2 hours and visualized without equipment(52). The RPA assay is considered rapid and extremely sensitive at point of care, as it uses a low-temperature isothermal reaction, reagents that do not require cold-chain logistics, and can be performed using only minimal equipment(42).

Table 4: Available rapid field based rapid molecular tests

Index Test	Details of Index Test	Image of test
LAMP: Loop mediated isothermal amplification	**Type of test**: Rapid, near patient, field laboratory **Sample**: Urine **Method of action**: LAMP assay amplifies a target DNA or RNA under isothermal conditions (60–65 °C), using a simplified commercially available LAMP buffer mix and reaction mix containing primers to attach to nucleotide sequences in the schistosome DNA fragment. End-point detection is visualised either by the naked eye or by adding colorimetric dyes. **Time to result:** 10 mins DNA extraction, 2 hours DNA amplification. **Field Applicability**: Minimal equipment needed, the test can be performed without DNA extraction, although field- based DNA extraction kits are also available. **Species specific:** Yes, detection of species-specific schistosome DNA in urine.	 LAMP fluorescence visible with naked eye
RPA: Recombinase polymerase amplification	**Type of test**: Rapid, near patient, field laboratory **Sample**: Urine **Method of action**: RPA assay is a DNA amplification technology able to amplify the repeat Dra1 *S. haematobium* genomic region, detected with lateral flow strips, or fluorescence. Commercial kit contains all reagents needed in the field. **Time to result:** Approximately 1 hour. **Field Applicability**: Minimal equipment needed, RPA reaction is initiated in test tubes at a field laboratory with hand shaking or centrifuging. Portable testing device measures fluorescence (Amplifire Isothermal Fluorometer) **Species specific:** Yes, detection of species-specific schistosome DNA in urine.	 RPA test with DNA reaction in test tubes

2.3.2.4 Mobile phone health devices

Osei *et al* (2021) published a systematic review on the application of mobile-linked point of care diagnostics in sub-Saharan Africa(53). mHealth technology is considered an emerging diagnostic tool especially for patients that have limited access to essential health care services. Digital health using devices such as mobile phones, tablets and mobile microscopes have demonstrated potential as a POC diagnostic tools. These devices developed as alternatives to microscopes for urogenital schistosomiasis diagnosis have been included in this review.

2.4 Summary.

Rapid tests are important health tools that increase the awareness of possible infection with *Schistosoma*. Early diagnosis leads to treatment and therefore prevention of chronic forms of schistosomiasis. Innovative rapid tests can pave the way for *Schistosoma* prevention and treatment programs in endemic areas of South Africa. At present, there are no programs in place, and schistosomiasis remains a neglected tropical disease, affecting millions of people in impoverished communities.

Innovative rapid tests are available as field applicable adaptions of laboratory tests and can give results from 15-20 minutes to 2 hours. Although many of the lateral flow serological tests still require serum, which needs a portable centrifuge, the antigen and molecular tests require urine which is easier to obtain. Field based molecular test such as LAMP and RPA are commercially available as a kit containing all the reagents and primers needed for DNA amplification in the field. Portable fluorescence testing devices or scanners and DNA extraction devices are needed for the UCP-CAA and RPA but considered to be field applicable in low resource laboratories.

CHAPTER 3: METHODS

3.1 Introduction:

This chapter describes the study design, setting and population that form the eligibility criteria of the studies chosen for this review. The data collection from four public medical databases and data extraction process from the eligible journal articles are explained. The management of collected data, and the methods used to approach the data analysis are explained in detail and follow the methods recommended in the Cochrane DTA handbook(27).

3.2 Study Design:

This is a systematic review of Diagnostic Test Accuracy (DTA). The DTA review summarises the evidence about test accuracy measured by specificity and sensitivity, using a reference standard. Methods of this study will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Diagnostic Test Accuracy (PRISMA-DTA) guidelines(54).

3.3 Study Setting

S. haematobium is essentially an infection acquired in sub-Saharan Africa, however DTA studies are performed in a variety of prevalence settings, either within the region of the endemic area, or in non-endemic areas where screening for migrants from Africa is performed. Study settings for schistosomiasis DTA studies are often screening centres, such as schools and community centres and clinics. In order to include a study population as close as possible to the natural population in an endemic area, all settings in Africa were included. Diagnostic accuracy studies done in non-endemic settings were included if travellers or migrants from endemic areas in Africa were tested. Results for the various rapid tests are reported generally for all settings, however, in order to determine test accuracy changes in various settings, sub-group analysis was done for prevalence setting based on the following categories, (according to WHO classification using parasitological methods):

-Endemic/high intensity: schistosomiasis prevalence of>50% in school aged children.

-Moderate intensity: schistosomiasis prevalence of >10% and <50% in school aged children.

-Low intensity (including post-elimination/low endemic): schistosomiasis prevalence <10% in school aged children.

-Non-endemic setting: Areas where schistosomiasis does not exist but where testing is done for non-immune travellers coming from an endemic area OR where testing is done on sub-Saharan migrants who harbour chronic schistosomiasis.

3.4 Study Population

People of all ages who are exposed to infection with *S. haematobium* infections in endemic regions, irrespective of healthcare, concomitant illness or geographic setting are included in this review. A sub-group analysis was performed based on age-group: Pre-school (<7 years), school aged children (8-15 years) and adult (>16 years) due to differences in intensity and chronicity of infection.

3.5 Data collection

The data collection process of a systematic review is concerned with test accuracy data presented in studies, their methodological detail, and the study findings. Major databases were systematically searched for eligible DTA studies using analogous search terms. These included PubMed, Global Health, ProQuest and the Web of Science.

3.5.1 Search strategy

Medical subject headings (MeSH) terms were used in the search strategy. The following search terms were used in all of the public databases searched:

(*Schistosoma haematobium*) AND (diagnostic test) AND (point of care test) OR (Urogenital schistosomiasis) AND (diagnostic test)) OR (*Schistosoma haematobium*) AND (rapid diagnostic test) OR (Diagnostic accuracy) AND (*Schistosoma haematobium*).

A second search strategy and article mining were done for individual index tests after the initial search to ensure that the totality of diagnostic performance studies is included. An example of search terms used is: ("*Schistosoma haematobium*") AND (“LAMP”), depending on each different index test.

3.5.2 Eligibility Criteria for the systematic review

3.5.2.1 Inclusion Criteria

3.5.2.1.1 Types of publications:

-Research journal articles or preprints published from January 2000 to July 2023 to ensure that all recent innovative advancements in diagnostics are included.

-All studies that are presented in the English language were included.

3.5.2.1.2 Types of studies

-Studies in which sensitivity and specificity or true-negatives (TNs), true-positives (TPs), false-positives (FPs), and false-negatives (FNs) can be calculated from the data presented to produce contingency tables for diagnostic accuracy and ROC analyses.

-Studies that analyse the performance accuracy of POC or rapid field applicable tests for *S. haematobium* infections.

-Primary observational studies that compare the results of an index tests to that of a reference standard (cross-sectional, prospective or retrospective cohort or diagnostic accuracy studies within randomized clinical trials). Case control studies were included if cases and controls are sampled from the same patient population.

3.5.2.1.3 Reference tests:

The reference tests used as comparator for this review are appropriate laboratory-based assays. Standard microscopy, composite tests, or statistical models using Latent Class Analysis (LCA) are included to provide an overall estimate of diagnostic accuracy.

-Urine microscopy methods should state if single or double/triple tests were performed, and if urine samples were concentrated by sedimentation, filtration or centrifugation techniques.

-Composite reference standards are accepted as a comparator to the index test, if the samples for the reference test are taken from the same population, and at the same time (within 24 hours).

-LCA models are accepted as a comparator to the index test. The results of two or more imperfect tests are used together to estimate an unmeasured 'true' infection status. Samples must be taken from the same population and at the same time.

-PCR as a highly sensitive laboratory- based test is accepted as a comparator in low prevalence settings or chronic disease manifestations.

-Data about the reference test should state sample preparation, number of samples per participant and the intensity of infection, as well as diagnostic threshold used to report a positive result.

Further sub-group analysis was performed for diagnostic accuracy differences based on different reference standards: 1) Composite of microscopy and laboratory-based tests such as PCR or ELISA 2) Duplicate and triplicate urine microscopy 3) LCA model 4) PCR only 5) Microscopy only.

3.5.2.1.4 Index Test:

The index test for this systematic review is any direct rapid field applicable or POC test that is sensitive to distinguish an infection of *S. haematobium* from a healthy non-infected individual. POC tests are defined as those that can be conducted at or near the clinical site with the aim to facilitate timely and cost-effective decision making(23). Rapid tests are defined as laboratory tests that use field-based kits for poor-resourced laboratories, and where the results are available within 2 hours.

-The results of index tests should be quantified as either positive or negative in both high and low intensity infections. Trace positives or negatives will not be included. Infection intensity levels are not considered in this review, as the focus is on whether a test can distinguish an infection, not its intensity.

-Index tests with a testing platform that is adapted for laboratories in low resource areas are included. Details about sample preparation methods and field equipment needed should be stated.

-For each index test, thresholds used for test positivity and factors relevant to test interpretation need to be stated.

-Meta-analyses will be attempted for the same POC/rapid test if there are 3 or more accuracy studies for the test. Tests with less than 2 studies will be analysed narratively.

3.5.2.2 Exclusion criteria

Studies will be excluded if they:

-Determine diagnostic accuracy for index tests that require extensive laboratory equipment or show a result in a time period of more than 2 hours. Example: PCR and ELISA antibody laboratory tests

-Examine diagnostic accuracy for *S. mansoni* only

-Do not state schistosomiasis prevalence

-Do not state the study setting with respect to endemicity

-Do not specify testing for active schistosomiasis

- Proof of concept studies and case-control studies using healthy controls from non-endemic areas are excluded to prevent bias and inflated specificity. Papers, non-research letters, commentaries or editorials are excluded.

3.6 Data management and analysis

3.6.1 Study selection

Software for systematic reviews from Rayyan (www.rayyan.ai) was downloaded and used as a reference manager for the systematic review. Studies were selected based on the pre-specified method outlined in the PRISMA_DTA guidelines(54), where a PRISMA flow diagram (Figure 3) was drawn up. From the database search described above, studies were identified, and duplicates were removed. Two reviewers independently screened the remaining studies, excluding those that did not keep to the eligibility framework. Exclusions were done in a step-by-step methodical manner. First by title, then by abstract, and then by full text screening. All included studies were uploaded onto the Rayyan reference manager software to improve the ease of independent review. Study abstracts were independently reviewed by each of the reviewers, after which merging their inclusions, and identifying and removing duplicates.

3.6.2 Data extraction:

Data was extracted and summarised in a data extraction table in Excel. The full text of each study was read to extract the necessary data.

The following data was extracted during the data extraction process:

- Study authors, publication year and journal
- Country where study was conducted.
- Setting in terms of endemic or non-endemic area
- Prevalence of schistosomiasis
- Study participants including age, sex, and patient selection.
- Reference standard used as a comparator, including number of samples per patient, and volume of urine/serum used.
- Index test- including name of manufacturer of test or equipment used, DNA extraction and amplification methods for molecular tests,
- Sample used for index test including preparation techniques.
- Threshold used to determine a positive test result.
- Numbers of TPs, FNs, FPs, and FNs entered into a 2x2 contingency table.

Contingency 2x2 tables containing FN, TN, TP and FP were drawn up for each DTA study based on the data extracted in the study. Any studies that had discrepancies between the reviewers were

screened and assessed for eligibility based on inclusion/exclusion criteria. Data collection methods recommended in the Cochrane DTA handbook were followed for identifying and structuring the statistical data needed for meta-analyses(55)

3.6.3 Data Analysis

From the data extraction table in Excell, studies were grouped according to index tests. The aim of the analysis is to compare the test accuracy for the included index tests and investigate heterogeneity between the studies selected for each index test. Methods for data analysis followed those recommended by the Cochrane Screening and Diagnostic Test Methods Group (SDTM)(56). The software recommended by Cochrane is RevMan 5.3 (training.cochrane.org), which is Cochrane's bespoke systematic review and meta-analysis tool. Raw data from the extraction process is imported into the RevMan software, which is able to generate forest plots and ROC curves, essential for the meta-analysis of DTA reviews.

3.6.3.1 Calculating Sensitivity and Specificity measures

In order to complete a 2x2 contingency table, the number of TPs, FNs, FPs, and FNs, and well as the total study population that was specifically tested for *S. haematobium* were attained from each study and entered into a 2x2 table. Specificity and specificity measures were calculated manually and compared with that reported in the study. TPs, FNs, FPs, and FNs were then entered into the RevMan 5.3 software, giving the sensitivity and specificity measures for each study, and determining the 95% confidence intervals.

3.6.3.2 Generating a forest plot and ROC curve for each index test

A forest plot was generated for each index test group to visually report the sensitivity and specificity values and confidence intervals. A receiver operator characteristic (ROC) plot was generated from the data producing a summary curve representing how the sensitivity and specificity of the included studies trade off with each other. The Review Manager 5.3 software package was utilised to generate the ROC and coupled forest plots required for DTA reviews. The forest plot and ROC curve for each index test was visually analysed for heterogeneity, which was reported as high, medium and low heterogeneity.

A ROC plot is a visual representation of the discriminative power of a test by plotting for each test, 1-specificity on the x-axis and sensitivity on the y-axis. The area under the ROC curve is a measure of the overall quality of a test where a curve within the top left corner of the ROC plot

represents a larger area under the curve, and therefore higher accuracy. More than one test can also be visually compared on one graph(27). A visual analysis of the ROC space with individual study plots can demonstrate the amount of heterogeneity between studies.

3.6.3.3 Meta-analysis of each index test

In order to complete the meta-analysis, data was imported from RevMan 5.3 to the statistical software STATA 17 (StataCorp. 2023. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.)(57) or SAS version 9.3(Copyright 2024 SAS Institute INC). The meta-analysis pools the sensitivity and specificity results for studies in each index test group, giving pooled accuracy measures for each index test. This will constitute the final diagnostic accuracy result for each index test. The meta-analysis could be performed for test types that had sufficient data points (3 or more studies with data for FN, TN, FP, TP in contingency tables), and those that did not demonstrate substantial heterogeneity in the ROC space. Tests with <3 studies were analysed narratively.

The statistical model selected for the overall analysis for each test depended how studies chose thresholds to determine positivity. For tests that have a single common threshold, the bivariate random-effects model as proposed by Reitsma was used for overall meta-analysis(58). For example, tests with lateral flow cassettes representing positivity with a colorimetric test and control band have a single threshold, and the bivariate model is then used. The statistical software STATA version 17 was used to perform bivariate modelling.

For tests that have the possibility of multiple thresholds, and variation in thresholds used in different studies, the hierarchical summary receiver operating characteristic model (HSROC) proposed by Rutter and Gatsonis(59) is used for overall meta-analysis. This model allows for variation in the parameters of accuracy and variation of thresholds between studies. HSROC modelling was performed using the statistical software SAS version 9.3 and models sensitivity and specificity indirectly. Therefore, average sensitivity and specificity were calculated using this model to account for between and within study variability.

3.6.3.4 Exploratory analysis for heterogeneity

Exploratory analysis was first performed using RevMan 5.3 to determine if a factor/co-variate is associated with test accuracy. The following variables were ascertained according to the information provided in each study: prevalence setting, age-group and reference test/comparator.

The forest plot groups the results according to the variables setting and reference test, and therefore a visual comparison of results is possible. ROC curves were generated for each of these 3 variables within each index test group and examined visually to see if the curves have too much variation in accuracy of the index test based on either prevalence setting, age, or reference test. Index tests with very heterogenous studies were further analysed using sub-group analysis techniques in STATA 17 to determine actual sensitivity and specificity measures for each sub-group (see 3.5.3.5).

3.6.3.5 Sub-group analysis

Investigations for heterogeneity was done visually by examining forest plots, and statistically by including co-variates in the bivariate or HSROC model. Subgroup analysis was performed for index tests that showed significant heterogeneity in relation to the co-variates: prevalence setting, age-group and reference test. For tests analysed with the bivariate model, we investigated whether these co-variates affect sensitivity and specificity. For tests analysed with the HSROC model, we investigated whether these co-variates graphically affect the shape of the curve by changing the parameters of the model. Differences in sensitivity/specificity were tabulated for each index test based on the subgroup evaluations.

3.6.4 Quality Appraisal

The methodological quality of the eligible studies was assessed by two reviewers who independently reviewed each study using a critical appraisal tool called Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2). The tool was used to assess the risk of bias for diagnostic accuracy studies(60). Disagreement between reviewers were resolved through consensus. QUADAS-2 is structured so that 4 key domains are rated in terms of risk of bias, and applicability to the research question. The domains are patient selection, reference standard, index test, and flow and timing. See Figure 4 and 5 for the risk of bias and applicability assessment for the included studies.

3.7 Ethical considerations

This study used the results of published papers and there was no interaction with participants or use of individual data. Therefore, there were no ethical implications for participants and no need to apply for ethics clearance. An ethics waiver certificate to certify that the research does not need ethics clearance was awarded by the University of Witwatersrand Human Research Ethics Committee, number W-CBP 230525-01.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter summarises the results of this DTA review. The results of the database search are presented in a PRISMA flow diagram (preferred reporting items for a systematic review and meta-analysis)(54). Reasons for exclusion of studies during screening and identification of studies are explained. The characteristics of the included studies are presented in a table, as well as the index tests identified as eligible rapid/POC tests for the diagnosis of urinary schistosomiasis. The methodological quality assessment of the included diagnostic accuracy studies using the QUADAS-2 tool(60) is presented. The pooled summary diagnostic performance results for the included index tests are presented in four sections: summary forest plots, summary ROC plot, summary results of the meta-analysis and the exploratory and sub-group analysis for heterogeneity between the studies. The Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy (Chapter 10) (27)was used as a guideline for the analysis and presenting of results.

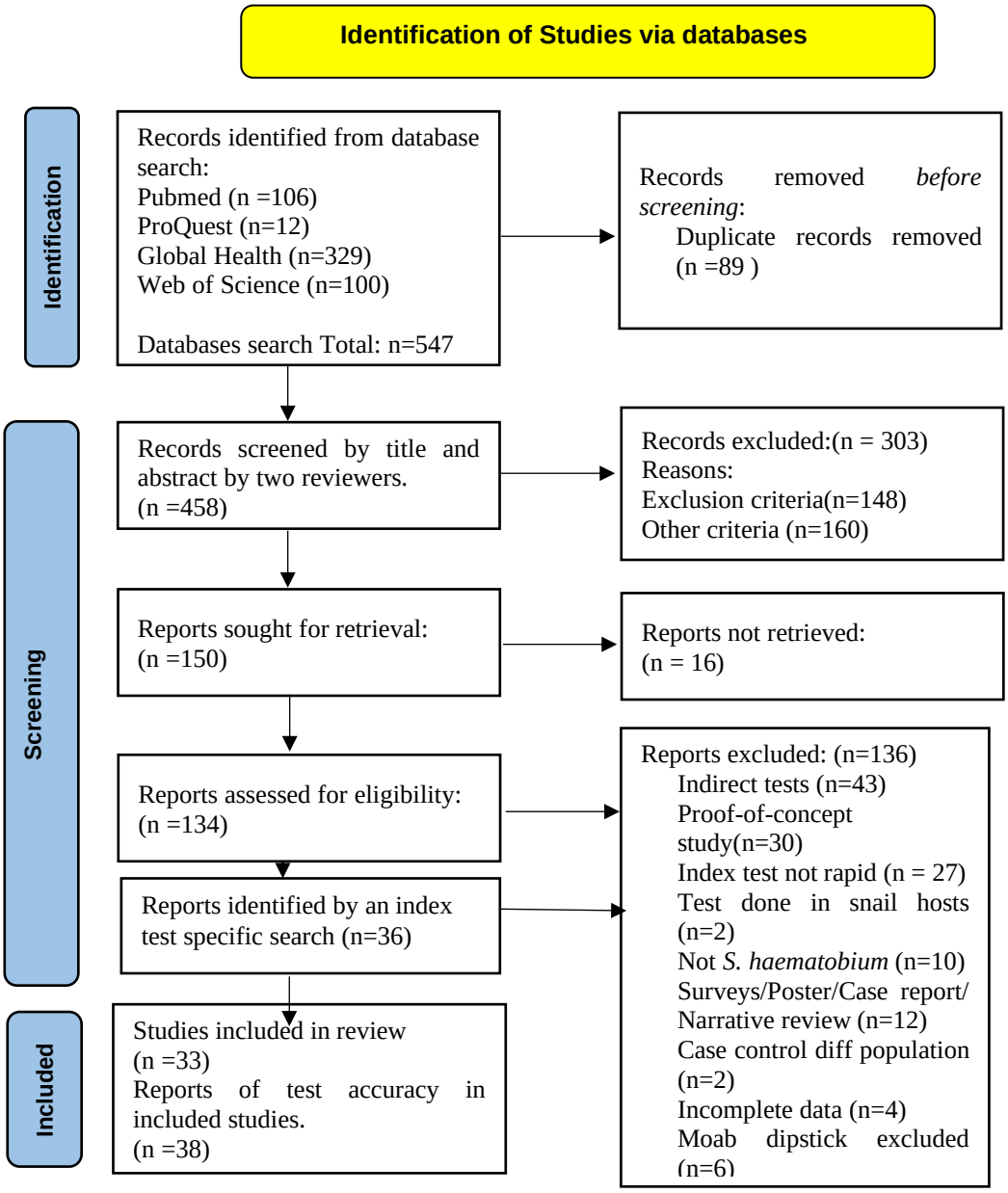
4.2 Results of the database search

The review gathered 547 peer reviewed studies from four public databases describing diagnostic performance of POC/rapid tests for *S. haematobium* of which 89 duplicates and 303 non-eligible studies were excluded by 2 reviewers using Rayyan reference manager. A second index test specific search was done after the initial search to identify more studies for individual tests, which produced 36 more studies.

Exclusion was systematically performed by two blinded reviewers using the systematic review software Rayyan (www.rayyann.ai). The first exclusion included studies that did not meet the eligibility criteria for diagnostic tests for *S. haematobium,* leaving 170 studies to be assessed for eligibility. Of these, 136 studies were excluded for the reasons outlined in the PRISMA diagram (Figure 3).

It was decided to exclude the rapid MoAb dipstick ELISA, as it is an outdated laboratory -based test developed in 1997. All indirect tests were excluded at this point, specifically urine reagent strips, urine proteins, ultrasound, and any morbidity markers. Proof of concept studies that determined accuracy measures from DNA or egg spiked urine in the laboratory, and not in the field were also excluded. Finally, a total of 134 studies were entered into analysis and assessed for eligibility.

Figure 3: PRISMA Flow diagram for identification of studies in systematic reviews



4.3 General description of studies reviewed

Ten direct rapid diagnostic tests were identified as eligible index tests through the systematic search for diagnostic accuracy studies. After data extraction and screening for inclusion criteria, 33 studies from which 38 accuracy reports were included in the analysis. Four studies analysed the diagnostic performance of more than 1 test, therefore 38 evaluations from 33 studies were included. The ten POC or rapid tests were identified as possible index tests for analysis and are presented in Table 5. The 33 studies represent 9361 participants. Seven of these index tests were entered into the meta-

analysis, as three index tests had each two or less diagnostic accuracy studies published, and therefore were analysed narratively.

Table 5 Diagnostic tests included in this review.

Test abbreviation	Test name	Rapid/POC	Studies	Participants
IHA	Indirect haemagglutination assay	Rapid	4	643
ICT-RDT	Immunochromatographic- rapid diagnostic test	Rapid	5	1369
SmCTF-RDT	*S.mansoni*-cercarial transformation fluid- RDT	Rapid	2	209
DDIA	Dipstick Dye Immuno Assay	Rapid	1	146
RDT-Sh	Rapid Diagnostic Test- *S. haematobium*	Rapid	1	150
POC-CCA	Point of Care- Circulating Cathodic antigen	POC	11	4093
UCP-LFCAA	Up Converting Phosphor Lateral Flow Circulating Anodic Antigen	Rapid	3	1278
LAMP	Loop Mediated Isothermal Amplification	Rapid	4	421
RPA	Recombinase polymerase amplification	Rapid	3	491
Mobile Microscope	Portable device connected to a cell phone	Rapid	4	561
TOTAL			38	9361

The characteristics of the included studies are presented in Table 6. All the studies included in the review were published within the period 2000 to 2023. We included 33 studies, of which 26 (78%) were carried out in Africa. Seven studies were performed in European countries on travellers, migrants, or asylum seekers from Africa. A total of nine studies were done in hospital or outpatient settings on suspected positive patients, and 24 (73%) were in field settings (village, school or rural district). The median prevalence of *S. haematobium* infection was 30.7% (range 2.5%-73%). Thirteen studies (39%) evaluated subjects for both *S. haematobium* and *S. mansoni* infection in migrants/travellers or co-endemic areas as schistosomiasis screening studies. Twenty studies focused on diagnostic tests for the diagnosis of urogenital schistosomiasis only. Most of the studies were cross-sectional studies (78%), either using urine/serum samples from previous studies (retrospective), or prospective, in field studies. Seven studies were case-control studies using samples for both groups from the same population. The studies are grouped by test type; serological, antigen, molecular and parasitological. Test thresholds that determine positivity are indicated next to each test. A positive test band threshold indicates lateral flow test cassette.

Table 6: Characteristics of included studies.

Author	Country	Participants	Study design	Setting	Prevalence	Sample type	Index test (Thresholds)	Reference test
SEROLOGICAL TESTS								
Zhang, 2020(47)	Zambia	148 school aged children aged 7 to 15	Cross sectional	Endemic	High (61%)	Serum	IHA (>1:1.20) DDIA (colour)	Double Urine Microscopy of filtered urine
Van Gool, 2002(45)	Netherlands /Belgium	25 adults with confirmed UGS (10 with Katayama fever). 283 controls with infections	Case control	Non-endemic	N/A	Serum	IHA (>1:1.60)	Microscopy Urine OR Clinical symptoms (Katayama fever).
Yameny, 2019(61)	Egypt	50 UGS positive urine samples, and 50 negative samples from adults.	Case control	Endemic	Moderate (15%)	Serum	IHA (>1.160)	Single Microscopy of urine either centrifuged, filtered or sedimentation
LeBlanc, 2021(62)	France	114 hospitalized migrant children aged 3-18 from sub-Saharan Africa with suspected schistosomiasis.	Prospective Cohort	Non-endemic	N/A	Serum	IHA (>1.160) ICT-RDT (+test band) POC-CCA (+test band	Latent Class Analysis. (WB, ELISA, IHA, microscopy, POC-CCA, ICT)
Mudenda, 2022(63)	Zambia	430 adults aged 18-50 years, inhabitants of Siyavonga district.	Cross sectional	Endemic	Low 4%	Serum	ICT-RDT (+test band)	Single microscopy of filtered urine
Beltrame, 2017(34)	Italy	343 archived frozen serum samples from asylum seekers and migrants all ages at	Retrospective, comparative study	Non endemic	N/A	Serum	ICT-RDT (+test band) POC-CCA (+test band)	Single microscopy of filtered urine
Longoni, 2021(39)	Italy	50 positive and 50 negative samples from Beltrame study banked samples	Case control	Non-endemic	N/A	Serum	ICT-RDT (+ test band)	Composite Reference: (Microscopy, CCA, ELISA, Schisto II Western Blot IgG and ICT IgG-IgM)
Houmsou, 2021(46)	Nigeria	374 participants all ages from a rural area in Nigeria	Cross sectional	Endemic	Moderate 33%	Serum	ICT-RDT (+test band)	Single microscopy of filtered urine
Sheele, 2013(64)	Kenya	160 school children aged 8-17 years recently experiencing blood in urine.	Cross sectional	Endemic	High 49%	Urine	RDT-Sh (colour change)	Single microscopy of filtered urine
Nausch, 2014(65)	Zimbabwe	91 School children with positive egg count for both/either *S. mansoni* or *S. hematobium*	Cross sectional	Endemic	High 58%	Serum	SmCFT-RDT (+test band)	Single microscopy of filtered urine

Author	Country	Participants	Study design	Setting	Prevalence	Sample type	Index test (Thresholds)	Reference test
Coulibaly, 2013(40)	Cote Ivoire	118 Preschool children (aged 3-6)	Cross sectional	Endemic	Low 4%	Finger-prick blood	SmCFT-RDT (+test band)	Double microscopy of filtered urine
ANTIGEN TESTS								
Knopp, 2015(66)	Zanzibar Tanzania	1740 school children aged 9-14 years, of which 1200 samples analysed.	Cross sectional	Endemic post MDA	Low <10%	Urine	UCP-LFCAA (>0.4pg/ml)	Latent Class Analysis (reagent strips and microscopy urine)
Hoekstra, 2021(67)	Belgium	34 Belgium travellers all ages with a PCR confirmed schistosomiasis.	Prospective cohort	Non endemic	N/A	Urine	UCP-LFCAA (0.3pg/ml)	Composite (Serum PCR, AWA IFA for IgM and SEA ELISA for IgG)
De Dood, 2018(50)	Tanzania	44 women aged 18-35	Cross sectional	Endemic	High 40-50%	Urine	UCP-LFCAA (0.3pg/ml)	Composite reference (microscopy and serum CAA)
Obeng, 2008(68)	Ghana	152 school children age 5-16	Case control	Endemic	Low 0-20%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Rubaba, 2018(49)	South Africa	420 children from 10 schools.	Cross sectional	Endemic	Moderate 40%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Sanneh, 2017(69)	The Gambia	1954 participants from 10 schools aged 7-14 years	Cross sectional	Endemic	Low 10%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Stothard, 2006	Zanzibar Tanzania	50 school children 25 positive, 25 negative for S. haematobium	Case control	Endemic	High 73%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Ashton, 2011(70)	Sudan	373 children aged 5-16 years	Cross sectional	Endemic	Moderate 26%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Ayele, 2008(71)	Ethiopia	206 school children	Cross sectional	Endemic	Low	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
El-Ghareeb, 2016(72)	Egypt	96 school children aged 5-12	Cross sectional	Endemic Post MDA	Low 5.3%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Robinson, 2021(73)	Madagascar	500 participants adults and children	Cross sectional	Endemic	High 47%	Urine	POC-CCA (+test band)	Single microscopy of filtered urine
Guillardie, 2021(74)	France	Travellers and migrants providing 143 urine samples	Retrospective cohort	Non endemic	N/A	Urine	POC-CCA (+test band)	Single microscopy of filtered urine

Author	Country	Participants	Study design	Setting	Prevalence	Sample type	Index test (Thresholds)	Reference test
MOLECULAR TESTS								
Lodh, 2017(75)	Ghana	86 participants aged 5-26 years	Cross sectional	Endemic		Urine	LAMP (fluorescence)	PCR Urine
Gandegesui,2015(41)	Spain	94 urine samples of whom 74 sub-Saharan migrant adults, and 16 non-endemic individuals	Prospective cohort	Non endemic	N/A	Urine	LAMP (fluorescence)	PCR Urine
Gandegesui, 2018(76)	Angola	172 school aged children (8-15 years)	Cross sectional	Endemic	High 50%	Urine	LAMP (fluorescence)	Single microscopy of filtered urine
Bayoumi, 2016(77)	Egypt	69 adults suspected cases at an out-patient clinic.	Cross sectional	Endemic	Moderate 44.9%	Urine	LAMP (fluorescence)	Single microscopy of filtered and centrifuged urine
Archer, 2022(78)	Zambia	Non-pregnant, sexually active women between the age of 18 and 35 years	Cross sectional	Endemic	Low 2.5%	Vaginal self-swab/ cervical lavage.	RPA-Sh (200mV)	PCR Cervical Lavage samples
Frimpong, 2021(79)	Ghana	135 urine samples chosen from 1290 participants resident in Kumasi all ages.	Case Control	Endemic		Urine	RPA-Sh (900mV)	Composite reference (RT-PCR Urine and single Microscopy of centrifuged urine)
Archer, 2020(42)	Zanzibar Tanzania	168 urine samples from school children aged 9–12 years. 100 proven positive 68 random controls	Case control	Endemic		Urine	RPA-Sh (200mV)	Single microscopy of filtered urine
PARASITOLOGICAL TESTS								
Bogoch, 2017(80)	Ghana	223 pupils (age 7–16), of whom 60 urine samples were chosen	Cross sectional	Endemic	High 73%	Urine	Mobile Microscope (egg detection)	Conventional microscopy single centrifuged urine
Ephrahim, 2015(81)	Ghana	49 Urine samples from school children ages 7-13 years.	Cross sectional	Endemic	High 69%	Urine	Mobile Microscope (egg detection)	Conventional microscopy single centrifuged urine
Coulibaly, 2016(82)	Cote Ivoire	226 children between 6 and 19 years.	Cross sectional	Endemic	Moderate 40%	Urine	Mobile Microscope (egg detection)	Conventional microscopy single filtered urine
Coulibaly, 2016(82)	Cote Ivoire	226 children between 6 and 19 years.	Cross sectional	Endemic	Moderate 40%	Urine	Mobile Microscope (egg detection)	

4.4 Quality Appraisal

The studies were rated as moderate to high overall methodological quality according to the QUADAS-2 tool for quality assessment of diagnostic accuracy studies(60). Figures 4 and 5 below show graphical representations of the percentage of studies that had risk of bias or applicability concerns. Figure 4 represents graphically the author's judgement regarding four domains for each included study, that is patient selection, index test, reference test, flow and timing (time between index test and reference test). Methods of patient selection were adequately described by over 70% of the studies. Overall, over 65% of the studies properly reported both index and reference tests and how they were conducted and interpreted. Over 30% of the studies were unclear about timing and interval between the index and reference test, with many non-commercial index tests being performed in laboratories in Europe or America.

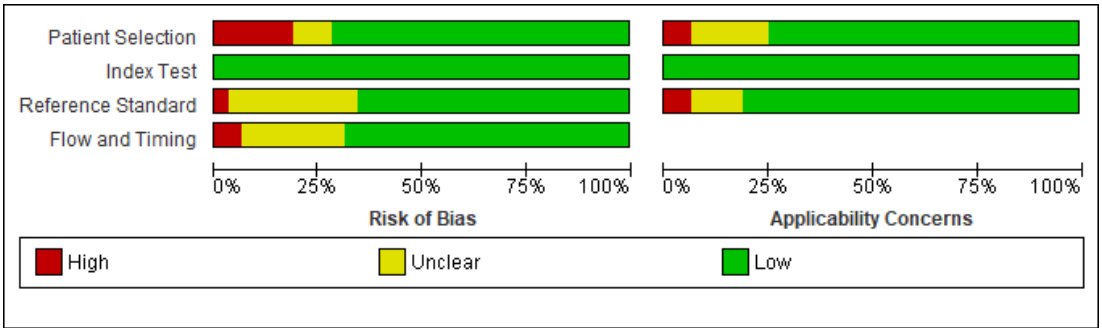


Figure 4: Risk of bias and applicability concerns graph: authors' judgements about each domain presented as percentages across included studies.

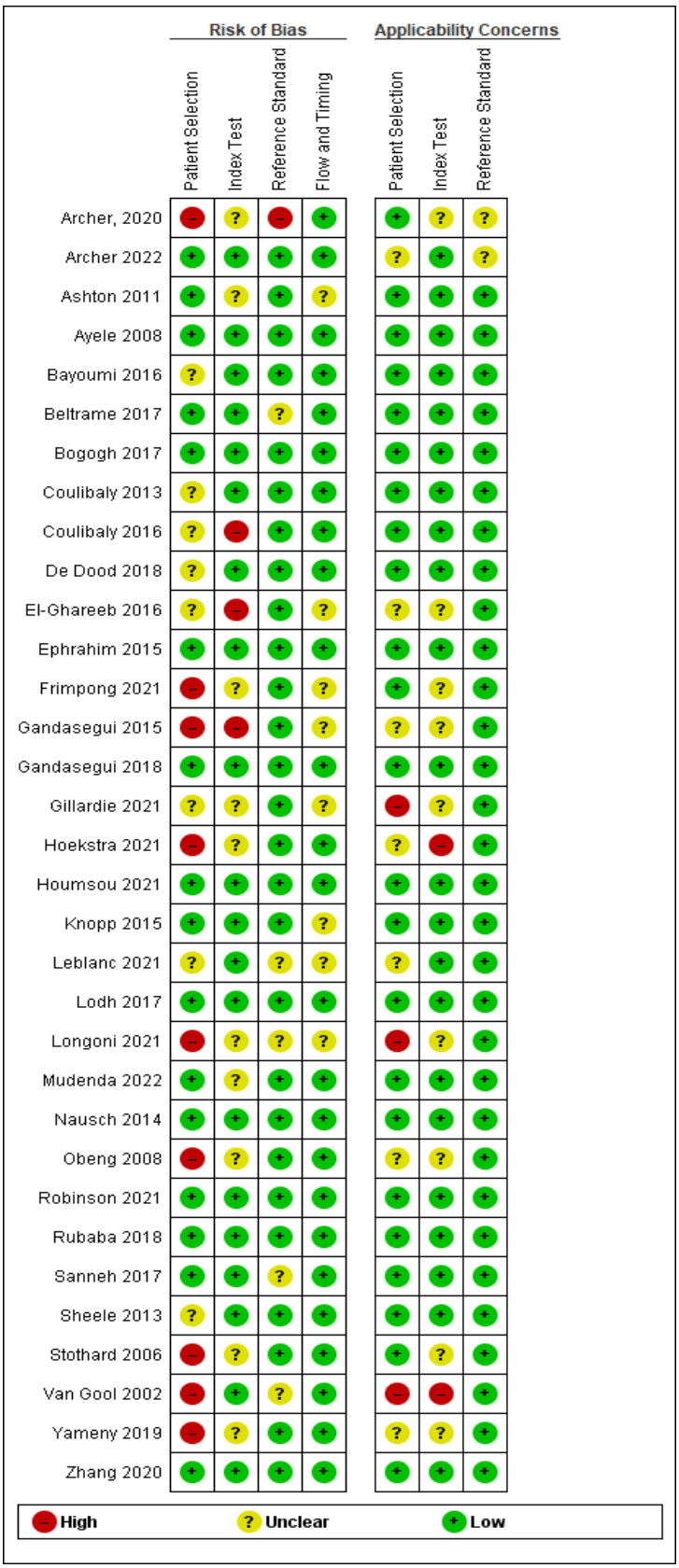


Figure 5 Risk of bias and applicability concerns summary: authors' judgements about each domain

4.5 Diagnostic performance of index tests for *S. haematobium*

This section presents the pooled diagnostic accuracy results for the 10 index tests reviewed. The summary estimates of sensitivity and specificity at the 95% CI region for all the included accuracy studies for rapid and POC tests are presented as summary forest plots and summary ROC graphs (Figure 6). Table 11 presents the summary diagnostic performance estimates for each index test attained from the meta-analysis, including the modelling methods used. In order to determine possible reasons for heterogeneity, an exploratory analysis of each forest plot and ROC plot was visually analysed and reported as high, medium or low heterogeneity. Further investigations into heterogeneity are presented as a sub-group analysis in Table 8, where the co-variates reference standard, age-group and prevalence of infection were added to the bivariate or HSROC model for each test.

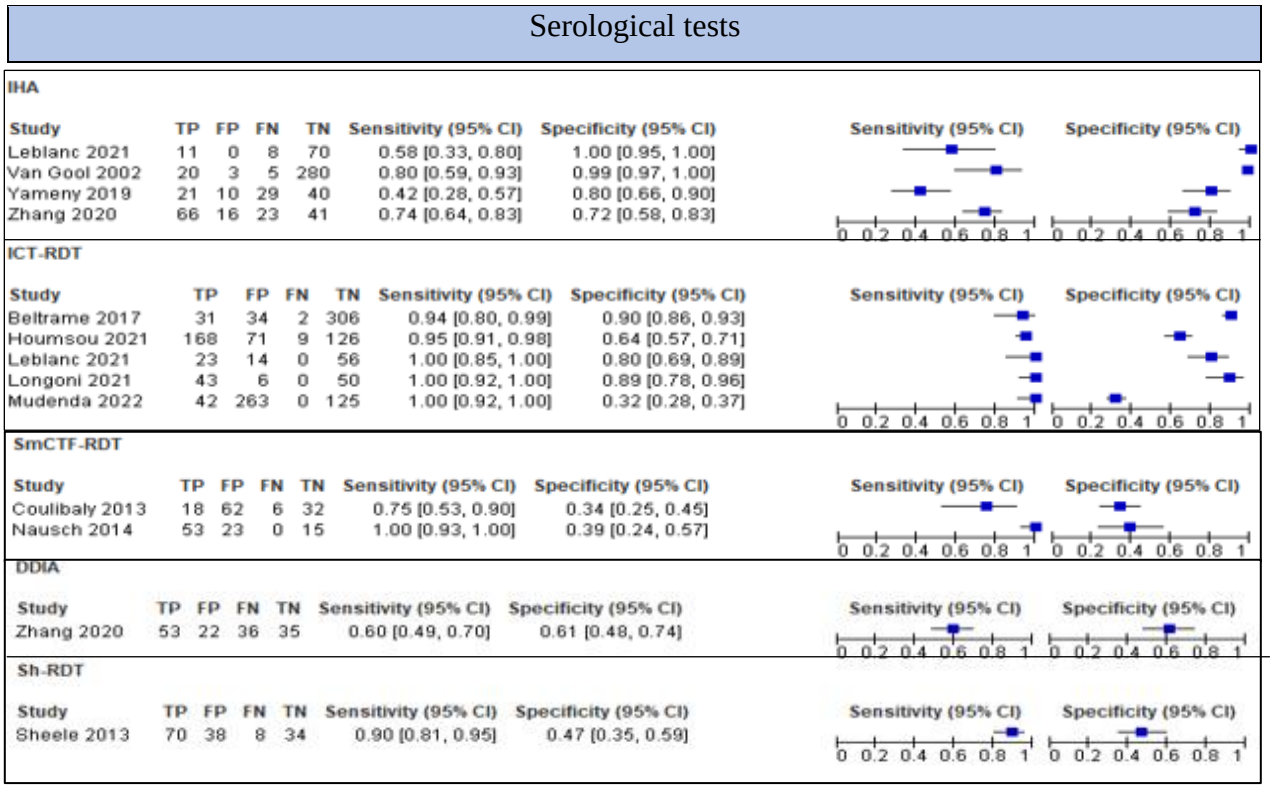
4.5.1 Forest plots for included index tests

Forest plots are presented for each of the ten index tests reviewed. The forest plot graphically represents the data from the 2x2 contingency table from which the estimates for sensitivity and specificity are calculated. For each study, the numbers of TP and FN in diseased individuals and FP and TN in non-diseased individuals are reported, as well as the summary estimates and confidence intervals for each individual study. The four rapid diagnostic methods compared in this review are serological tests, antigen tests, molecular tests and mobile-based microscopy.

4.5.1.1 Serological tests

The forest plots of the five immunological tests are presented in Table 7, and can be compared visually, where large confidence intervals are observed for IHA and SmCTF-RDT. Of the 13 studies included in this group, 11 were referenced by parasitological urine filtration methods, and two studies were referenced by LCA and composite reference tests.

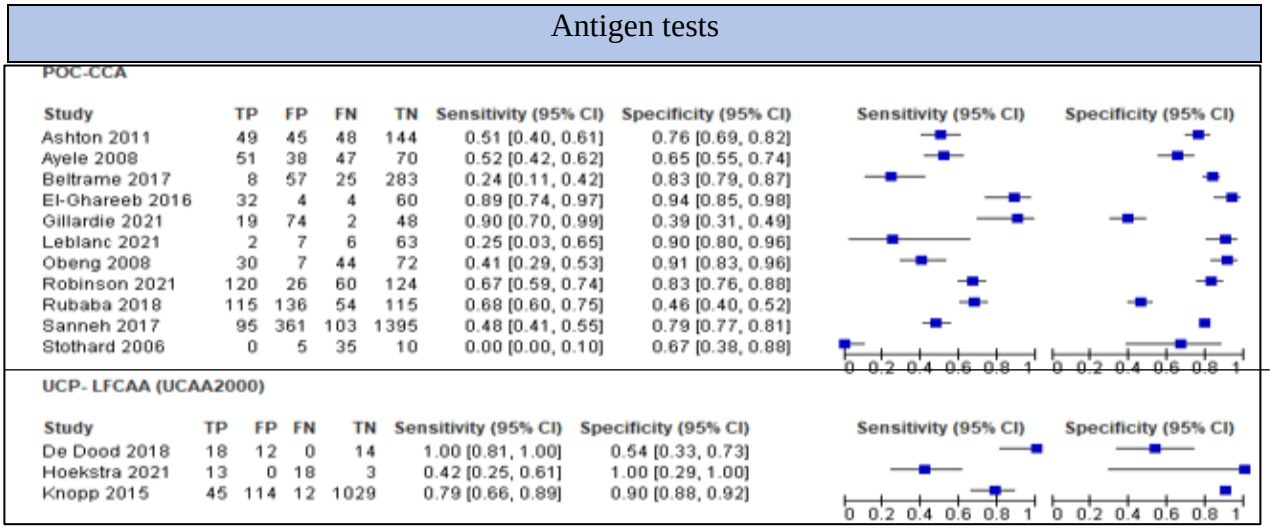
Table 7: Forest plot for sensitivity and specificity of serological tests for urogenital schistosomiasis



4.5.1.2 Antigen tests

The POC-CCA test was referenced by single microscopic examination of urine, and reveals a large variation in sensitivity, and large confidence intervals with low sensitivity and specificity values. The UCP-LFCAA however, was referenced by PCR, composite and LCA methods, and reveals high heterogeneity with large confidence intervals.

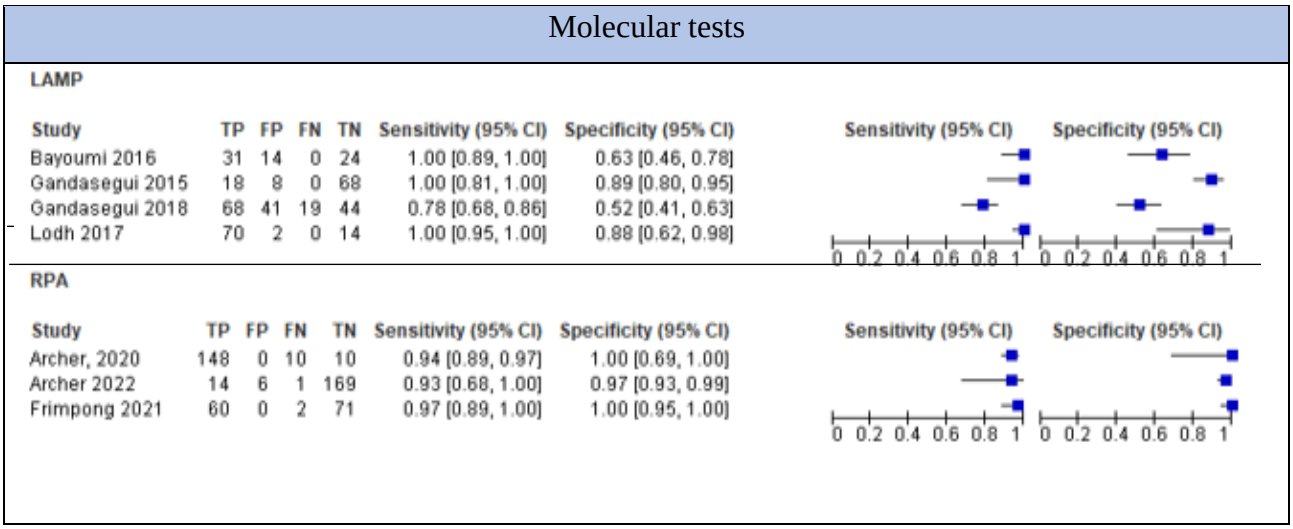
Table 8: Forest plot of sensitivity and specificity of antigen tests for urogenital schistosomiasis



4.5.1.3 Molecular tests

The forest plot LAMP and RPA reveal both reveal moderate heterogeneity and high sensitivity with some studies showing large confidence intervals. Of the 7 studies, 5 were referenced by PCR, while 2 studies were referenced by microscopy which implies that these rapid tests were highly comparable to laboratory-based molecular tests.

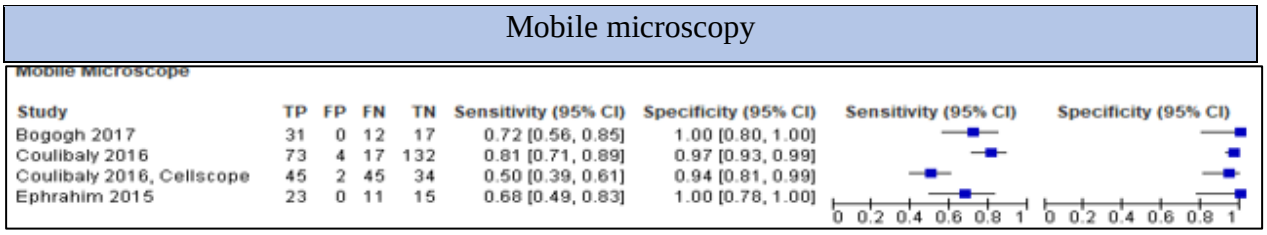
Table 9: Forest plots of sensitivity and specificity of rapid molecular tests for urogenital schistosomiasis



4.5.1.4 Mobile microscopy

Two mobile phone-based microscopes, the Cellscope and a portable microscope were to referenced against laboratory-based microscopy of filtered urine. The 4 studies reveal moderate heterogeneity for sensitivity, but a high specificity overall.

Table 10: Forest plot of sensitivity and specificity for mobile microscopy



4.5.2 Summary ROC curve for included index tests

The summary ROC plot in Figure 7 shows that the RPA molecular test has the highest diagnostic performance, as the RPA curve (shown as a pink line) is on the highest far left corner of the ROC plot. LAMP, ICT-RDT and mobile microscopy are grouped as the second highest diagnostic accuracy, while POC-CCA and DDIA have the poorest diagnostic accuracy.

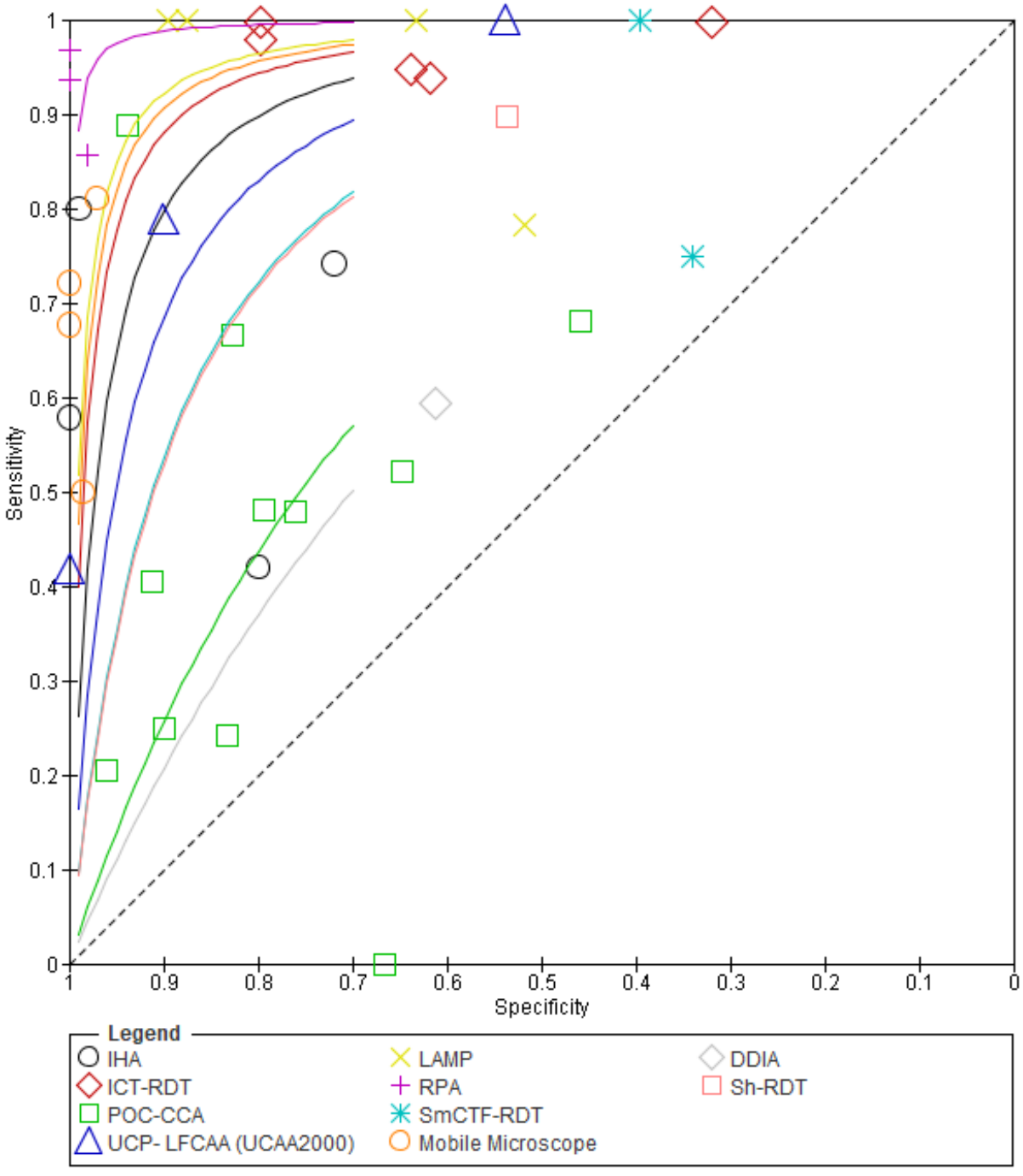


Figure 6: Summary ROC curve for all included index tests: higher accuracy tests are near top left corner

4.5.3 Meta-analysis results reporting summary sensitivity and specificity values

Meta-analysis was performed using STATA 17 (StataCorp. 2023. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC) (57) or SAS 9.3 statistical software (Copyright 2024 SAS Institute), using methods outlined in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy Chapter 10(27). Diagnostic thresholds determining positivity for index tests are reported for each study in Table 6. Index tests with same threshold were analysed using the bivariate model in STATA 17. Index tests with different thresholds were analysed using the HSROC model in SAS 9.3. The results of the meta-analysis are presented as an overall summary point for sensitivity and specificity with 95% confidence intervals.

Table 11: Summary results for rapid and POC index tests that directly diagnose urogenital schistosomiasis for individuals infected in African settings

Index test type	Number of studies	Pooled number of participants	Number with schistosomiasis (%)	Method of Analysis	Sensitivity % (95% CI)	Specificity % (95% CI)
ANTIBODY TESTS						
ICT-RDT	5	1369	318 (23.2)	Bivariate model	96.3% (87.7-99.6)	64.1 % (52.5-88.8)
IHA	4	643	183 (28.4)	HSROC model	64.8% (48.0-78.6)	95.6% (71.7-99.5)
RDT-Sh	1	150	78 (52.0)	Narrative analysis	90.0% (81.0-95.0)	54.0% (42.0-65.0)
SmCTF-RDT	2	209	77 (36.8)	Narrative analysis	98.4% (7.70-99.9)	36.7% (27.4-47.6)
DDIA	1	146	89 (60.9)	Narrative analysis	60.0% (49.0-70.0)	61.0% (48.0-74.0)
ANTIGEN TESTS						
POC-CCA	11	4093	949 (23.2)	Bivariate model	48.9% (27.7-70.4)	77.3% (66.0-87.5)
UCP-LFCAA	3	1278	106 (8.3)	HSROC model	87.4% (27.6-99.2)	86.4% (60.5- 96.3)
MOLECULAR TESTS						
LAMP	4	421	206 (48.9)	Bivariate model	99.9% (8.9-100)	76.2% (57,2-87.5)
RPA	3	491	235 (47.8)	HSROC model	94.1% (90.0-96.0)	96.6% (96.0-99.0)
PARASITOLOGY						
Mobile microscopy	4	561	257 (45.8)	Bivariate model	68.4% (54.9-73.4)	98.1% (98.5-99.2)

The molecular test RPA had the highest overall accuracy (sensitivity= 94.1%, specificity= 96.6%), displaying the best trade-off between sensitivity and specificity. LAMP and UCP-LFCAA can be grouped as the second highest diagnostic accuracy, based on the meta-analysis with sensitivity=99.9%, specificity 76.2% for LAMP, and sensitivity=87.4% and specificity=86.4% for UCP-LFCAA.

Mobile microscopy (sensitivity=68.4%, specificity=98.1%) and the serological screening test ICT-RDT (sensitivity=96.3%, specificity=64.1%) come close in accuracy after LAMP for *S. haematobium* diagnosis, where ICT-RDT is more sensitive and mobile microscopes are more specific. Tests with a moderate accuracy are the IHA, the UCP-LFCAA antigen test, the Sm-CTF and Sh-RDT serological tests, where further research could provide answers for their use as rapid tests in endemic areas.

Our overall results indicate that the DDIA (sensitivity =60.0%, specificity=61.0%) and POC-CCA (sensitivity =48.9%, specificity=77.3%) index tests have low diagnostic accuracy for *S. haematobium* and may not be suitable for prevalence mapping or estimation of infection. Both tests miss many infections that are identified by laboratory-based tests and are therefore not useful in practice.

Overall, RPA is the most suitable test for prevalence estimation in both high and low endemic settings, as well as for estimation of elimination of transmission of *S. haematobium*. LAMP is a highly sensitive test and can be used for screening and mapping, but our results show that the LAMP test is less specific because the studies chosen used a poor reference standard thereby compromising their results.

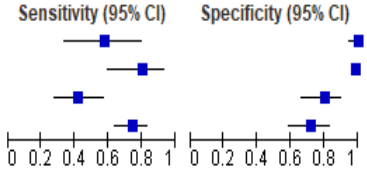
4.5.4 Exploratory analysis for heterogeneity of results

Tests with more than 3 studies were explored visually for possible reasons for the heterogeneity of results. Coupled forest plots including covariates and summary ROC plots were visually assessed. ROC plots for the co-variates age-group, prevalence and reference test were drawn up for each index test. The most heterogenous co-variate ROC plot is reported, but the full exploratory analysis of co-variate subgroups is shown in Appendix D.

4.5.4.1 Exploratory analysis for IHA

Possible heterogeneity for IHA accuracy results were explored for the co-variates prevalenece setting, reference standard and age group

Study	TP	FP	FN	TN	Age group	Reference	Prevalence	Sensitivity (95% CI)	Specificity (95% CI)
Leblanc 2021	11	0	8	70	SAC	LCA	Non-endemic	0.58 [0.33, 0.80]	1.00 [0.95, 1.00]
Van Gool 2002	20	3	5	280	Adults	Microscopy	Non-endemic	0.80 [0.59, 0.93]	0.99 [0.97, 1.00]
Yameny 2019	21	10	29	40	Adults	Microscopy	Low Endemic	0.42 [0.28, 0.57]	0.80 [0.66, 0.90]
Zhang 2020	66	16	23	41	SAC	Microscopy	High Endemic	0.74 [0.64, 0.83]	0.72 [0.58, 0.83]



SAC= School aged children. LCA=Latent Class Analysis

Figure 7: Forest plot of sensitivity and specificity for IHA rapid diagnostic test including co-variates

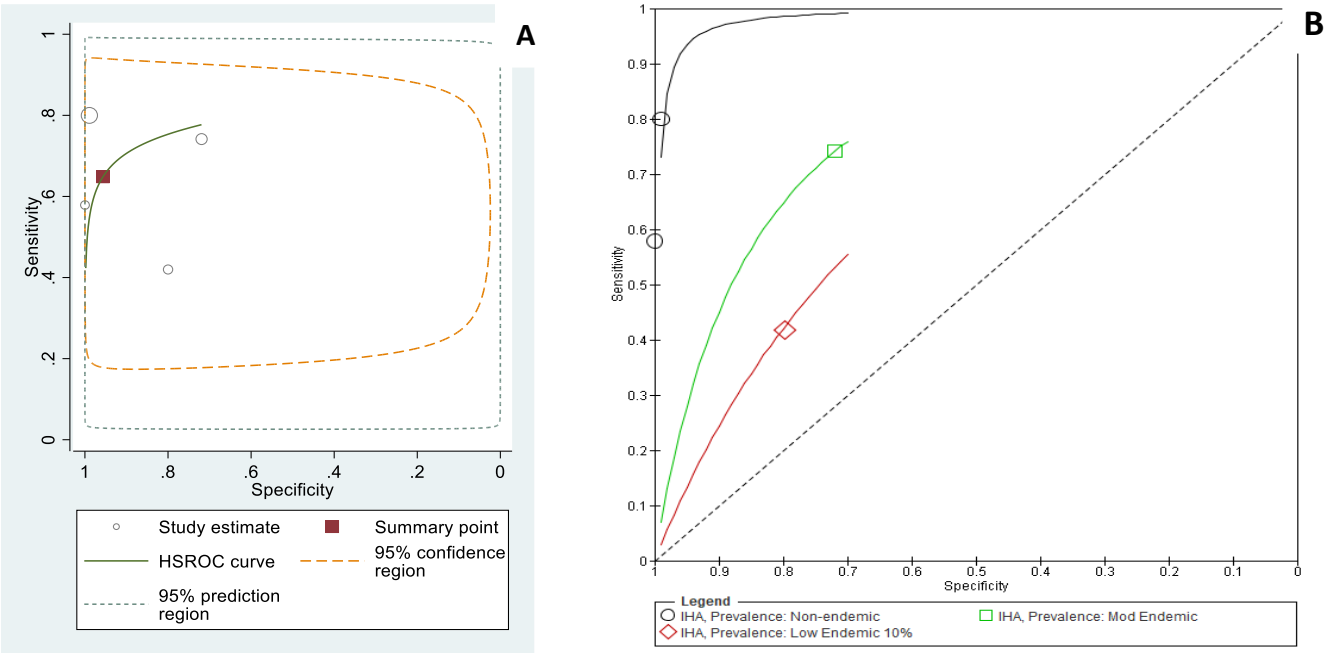


Figure 8: Summary ROC plot for IHA (A) and ROC plot for subgroup: Prevalence setting (B)

Visual assessment of heterogeneity for IHA: High

Figure 7 shows large variation and high heterogeneity for sensitivity.

The large 95% confidence region of the summary ROC plot for IHA in Figure 8(A) indicates that there is low accuracy of the summary point, and high heterogeneity.

Figure 8 (B) shows that the IHA has low accuracy in low endemic settings, implying that the various prevalence settings may be the reason for heterogeneity in the accuracy results for the IHA test.

4.5.4.2 Exploratory analysis for ICT-RDT

Heterogeneity in accuracy results for ICT-RDT are presented as a forest plot with co-variates, and summary ROC plot

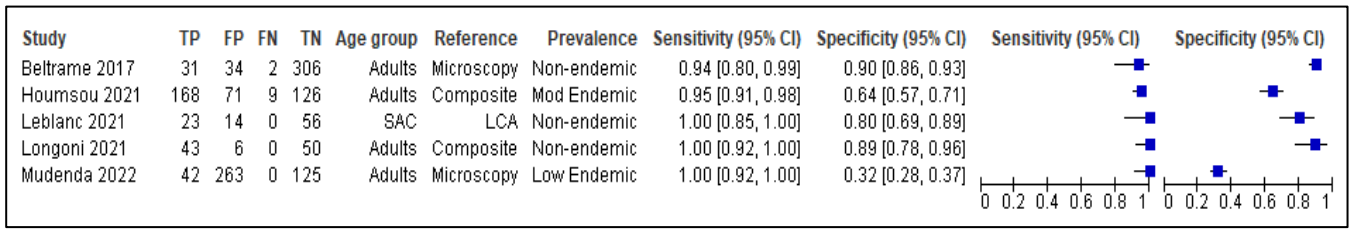


Figure 9: Forest plot of sensitivity and specificity for ICT-RDT rapid diagnostic test including co-variates

SAC= School aged children. LCA=Latent Class Analysis

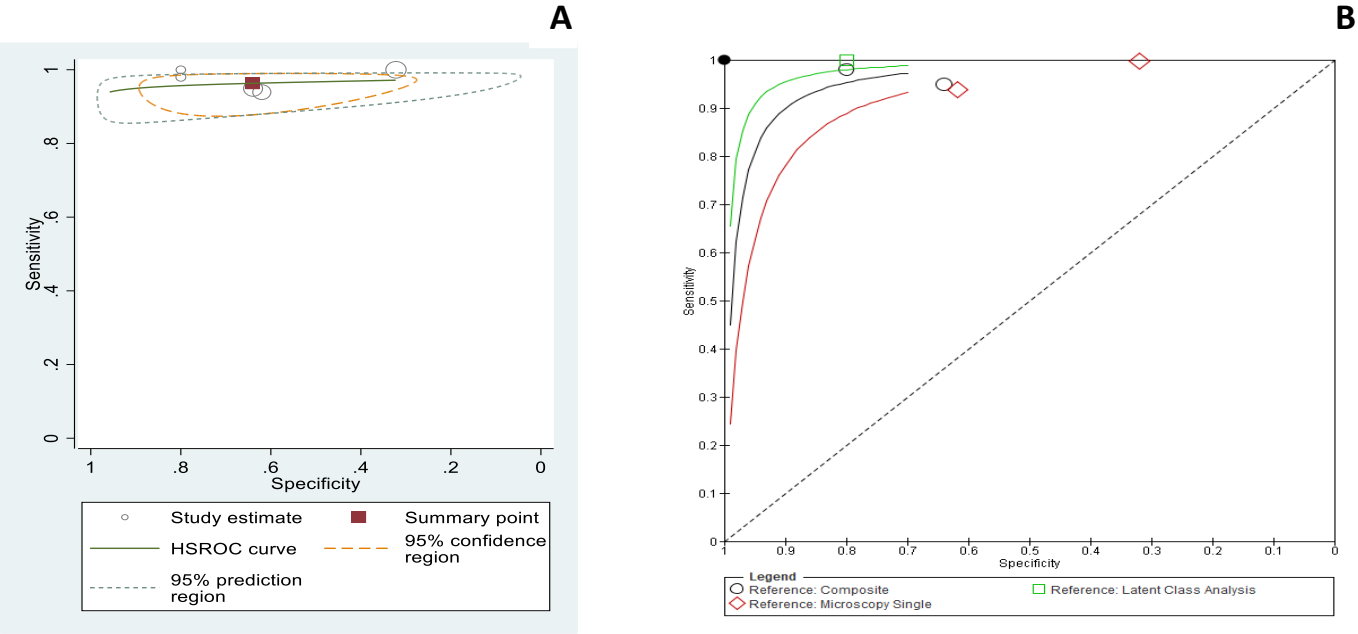


Figure 10: Summary ROC plot of ICT-RDT (A) and ROC plot for ICT-RDT: sub-group Reference test (B)

Visual assessment of heterogeneity for ICT-RDT: Moderate

The forest plot shows low variation in sensitivity, but higher variation in specificity, where endemic settings have lower accuracy.

The ROC plot has a small 95% confidence region, and therefore improved summary point accuracy.

The quality of the reference test affects the quality of the results where both the LCA and the composite reference show superior diagnostic performance compared to the single microscopy. The ICT-RDT has a high sensitivity, and its specificity is therefore inaccurately portrayed by the usual gold standard using single microscopy of filtered urine.

4.5.4.3 Exploratory analysis for POC-CCA

Heterogeneity in accuracy results for POC-CCA are presented as a forest plot with co-variates, and summary ROC plot.

Study	TP	FP	FN	TN	Age group	Reference	Prevalence	Sensitivity (95% CI)	Specificity (95% CI)
Ashton 2011	49	45	48	144	SAC	Microscopy	Mod Endemic	0.51 [0.40, 0.61]	0.76 [0.69, 0.82]
Ayele 2008	51	38	47	70	SAC	Microscopy	Low Endemic	0.52 [0.42, 0.62]	0.65 [0.55, 0.74]
Beltrame 2017	8	57	25	283	All ages	LCA	Non-endemic	0.24 [0.11, 0.42]	0.83 [0.79, 0.87]
El-Ghareeb 2016	32	4	4	60	SAC	Microscopy	Low Endemic	0.89 [0.74, 0.97]	0.94 [0.85, 0.98]
Gillardie 2021	19	74	2	48	All ages	Composite	Non-endemic	0.90 [0.70, 0.99]	0.39 [0.31, 0.49]
Leblanc 2021	2	7	6	63	SAC	LCA	Non-endemic	0.25 [0.03, 0.65]	0.90 [0.80, 0.96]
Obeng 2008	30	7	44	72	SAC	Microscopy	Low Endemic	0.41 [0.29, 0.53]	0.91 [0.83, 0.96]
Robinson 2021	120	26	60	124	All ages	Microscopy	High Endemic	0.67 [0.59, 0.74]	0.83 [0.76, 0.88]
Rubaba 2018	115	136	54	115	SAC	Microscopy	Mod Endemic	0.68 [0.60, 0.75]	0.46 [0.40, 0.52]
Sanneh 2017	95	361	103	1395	SAC	Microscopy	Low Endemic	0.48 [0.41, 0.55]	0.79 [0.77, 0.81]
Stothard 2006	0	5	35	10	SAC	Microscopy	High Endemic	0.00 [0.00, 0.10]	0.67 [0.38, 0.88]

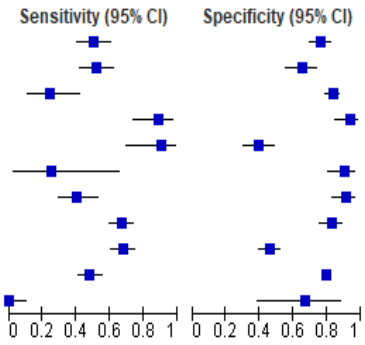


Figure 11: Forest plot of sensitivity and specificity of POC-CCA including co-variates

SAC= School aged children. LCA=Latent Class Analysis

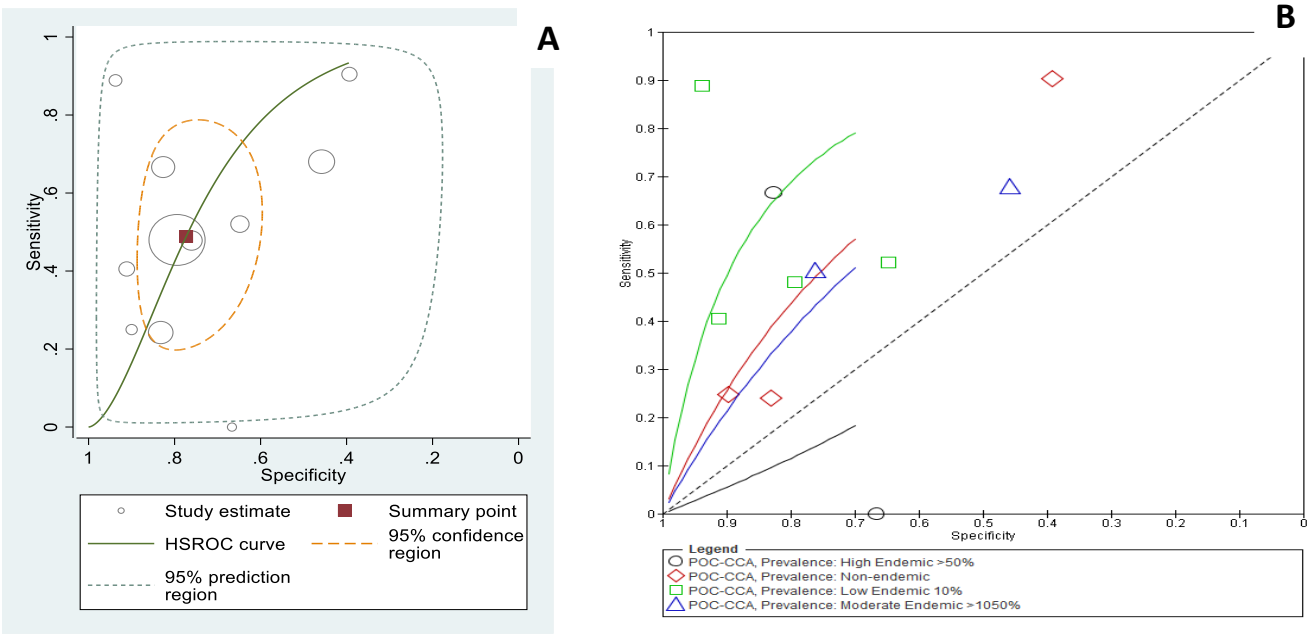


Figure 12: Summary ROC plot for POC-CCA (A) and ROC plot for POC-CCA: subgroup prevalence setting(B)

Visual assessment of heterogeneity for POC-CCA: High

Sensitivity values in the forest plot are visually highly heterogenous. Sampling bias and incorrect reporting occurred in 2 studies. Various prevalence settings may cause heterogeneity.

The summary ROC plot has a large 95% confidence and prediction region, as well as one study with a large sample size that influenced the summary estimate.

Visually it can be seen that the studies that were performed in a low prevalence endemic area had a better sensitivity than other settings. It appears that POC-CCA is not suitable for screening of migrants/travellers for *S. haematobium* as well as not suitable for endemic areas.

4.5.4.4 Exploratory analysis for UCP-LFCAA

Heterogeneity in accuracy measures for UCP-LFCAA is explored in Figure 13 and 14

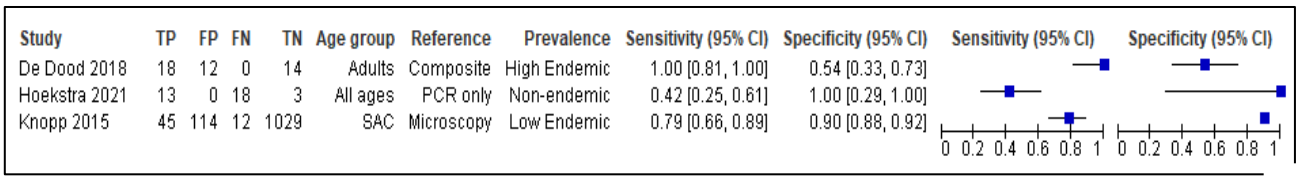


Figure 13: Forest Plot of sensitivity and specificity for UCP-LFCAA including co-variates

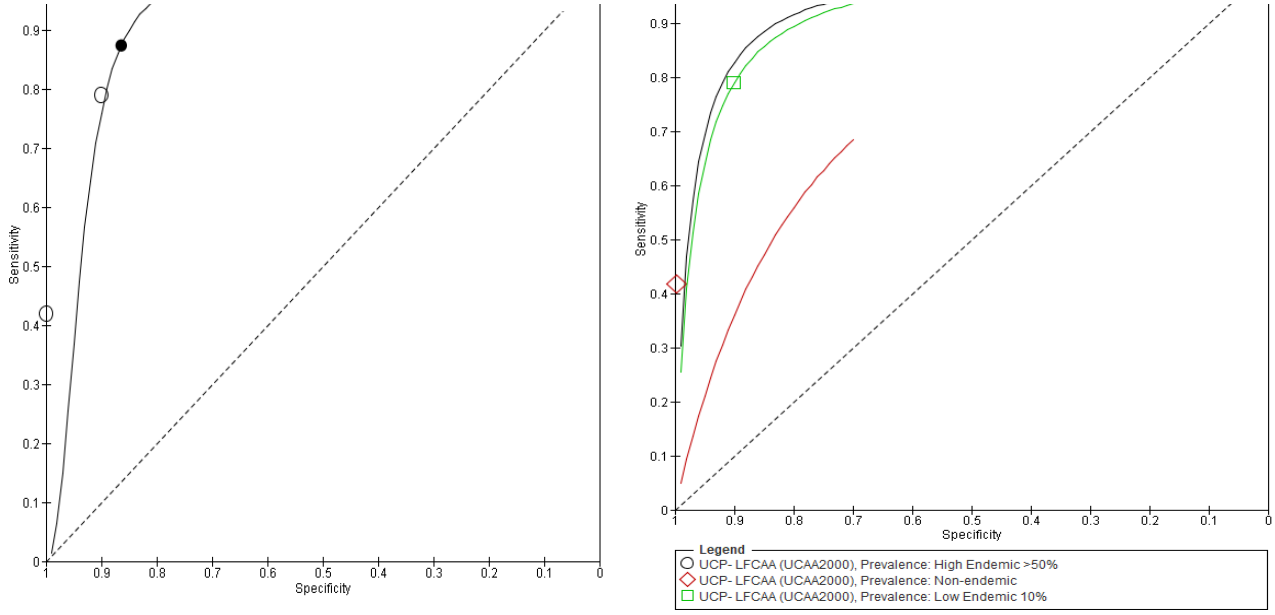


Figure 12: Summary ROC plot for UCP-LFCAA (A) and ROC plot for subgroup prevalence setting (B)

Visual assessment of heterogeneity of UCP-LFCAA: High

The forest plot exhibits highly heterogeneous results for the 3 very different studies, possibly due to comparing acute infections (Hoekstra) and chronic/low intensity infections (De Dood and Knopp).

The ROC plot reveals that the summary point for each of the 3 tests are widely spaced on the ROC space, indicating heterogeneous accuracy results.

Endemic settings both high and low are well suited for the UCP-CAA test. However, the test performs poorly for the study performed in non-endemic settings for screening of acute stage schistosomiasis in travellers.

4.5.4.5 Exploratory analysis for LAMP

Heterogeneity in accuracy results for LAMP are presented as a forest plot with co-variates, and summary ROC plot

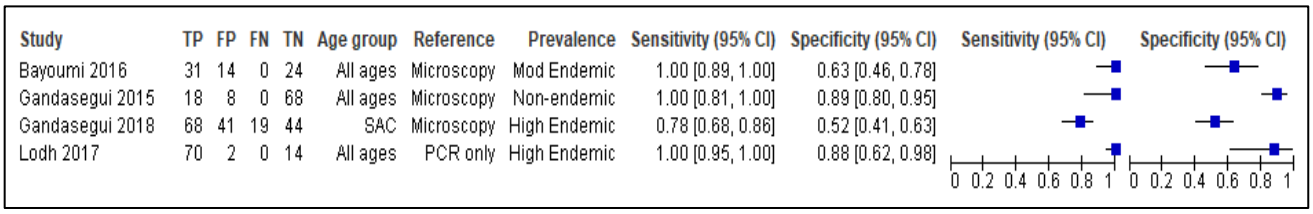


Figure 14: Forest plot of sensitivity and specificity of LAMP including co-variates

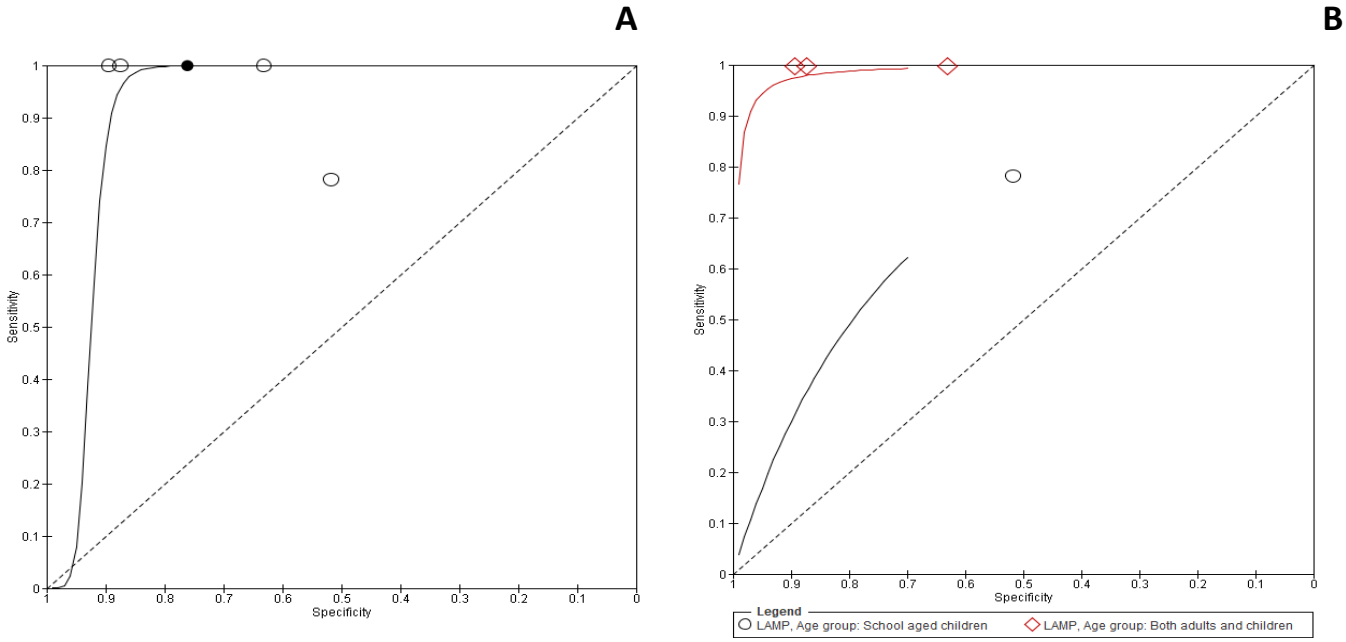


Figure 15: Summary ROC plot for LAMP (A) and ROC plot for LAMP subgroup: age group (B)

Visual assessment of heterogeneity: Moderate

The forest plot reveals heterogeneity for specificity, where one endemic study has lower specificity. Age group subgroup analysis for LAMP reveal that school aged children reveal far lower accuracy than the LAMP assay performed in adults. Further analysis revealed that the study by Gandagesui 2018 (76), in school children used an imperfect field- based DNA extraction method (rapid heat LAMPellet assay), while all other studies had improved DNA extraction methods.

4.5.5 Sub-group analysis

Due to a large variability in results from individual studies, and high heterogeneity observed in the exploratory analysis, a subgroup analysis was performed to determine reasons for heterogeneity in accuracy measures. The co-variates reference standard, age-group and prevalence of infection were

added to the bivariate or HSROC model for each test. Investigation was done to determine if any of these co-variates affect the parameters of the model in terms of accuracy (bivariate model) and visual changes in the shape of the ROC graph (for HSROC model). Although there may be more co-variates that cause heterogeneity, such as study design and infection intensity, there were not enough studies to investigate these co-variates. Table 12 summarises the results of the sub-group analyses for each test. Where there were sufficient numbers of studies (>2 studies per subgroup), meta-analysis was performed. Where there were one or two studies, the actual results of the study are presented.

Comparisons can be made between the overall accuracy and subgroup accuracy for each index test.

Table 1211: Subgroup analysis for individual index tests comparing overall accuracy results with subgroups accuracy results.

	Co-variate	**Sub- group (studies)**	**Participants (n)**	**Sensitivity (%) (95% CI)**	**Specificity (%) (95% CI)**
ANTIBODY TESTS					
ICT-RDT: Overall accuracy (5 studies)			**N=1369**	**96.3 (87.7-99.6)**	**64.1 (52.5-88.8)**
Subgroup analysis:	**Reference standard**	LCA / composite (3)	566	96.0 (92.6-97.8)	62.5 (55.9-68.7)
		Single microscopy (2)	803	99.1 (43.1-99.9)	46.7 (27.1-67.5)
	Prevalence setting	Non-endemic (3)	565	97.7 (89.1-99.6	73.4 (61.4-82.7)
		Endemic (2)	804		
	Age group	Adults >18 years (4)	1276	96.3 (92.5-98.2)	59.2 (41.4-74.8)
		School aged children (1)	93	100 (85.0-100)	80 (69.0-89.0)
IHA: Overall accuracy (4 studies)			**N=643**	**64.8 (48.0-78.6)**	**95.6 (71.7-99.5)**
	Reference stand	LCA (1)	89	58.0 (33.0-80.0)	100 (95.0-100)
		Single microscopy (3)	554	67.3 (45.3-83.6)	90.8 (61.2-98.4)
	Prevalence Setting	Non-endemic (2)	397	70.3 (51.5-84.1	99.3 (96.4-99.8)
		Endemic (2)	246	59.5 (35.8-79.5)	76.1 (65.1-84.5)
	Age group	School aged children (2)	235	66.5 (49.1-80.3)	98.6 (11.1-99.8)
		Adults >18 years (2)	408	62.6 (31.5-86.0)	95.2 (67.0-99.5
ANTIGEN TESTS					
POC-CCA: Overall accuracy (11 studies)			**N=4093**	**48.86 (27.7-70,4)**	**77.3 (66.0-85.6)**
	Reference standard	LCA/Composite (3)	594	63.8 (11.8-95.9)	70.4 (26.7-93.9)

	Co-variate	Sub- group (studies)	Participants (n)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
		Single microscopy (8)	3499	48.9 (25.1-73.1)	0.77 (65.1-86.4)
	Prevalence setting	Non-endemic (3)	594	46.0 (10.3-86.4)	74.5 (45.5-91.1)
		Endemic (8)	3499	58.5 (37.3-77.4)	84.6 (70.3-92.7)
	Age group	Adults>18 years (3)	846	66.8 (27.0-91.6)	71.1 (45.8-87.8)
		Children (8)	3247	43.5 (20.2-70.1)	79.1 (66.1-87.9)
UCP-LFCAA: Overall accuracy (3 studies)			**N=1278**	**87.4 (27.6-99.2)**	**86.4 (60.5- 96.3)**
	Reference standard	Composite (1)	44	100 (81.0-100)	54 (33-73)
		PCR (1)	34	42 (25.0-61.0)	100 (29-100)
		Microscopy (1)	1200	79 (66.0-89.0)	90 (88.0-92.0)
	Prevalence setting	Endemic (2)	1244	97.1 (20.5-99.9)	77.5 (45.1-93.5)
		Non-endemic (1)	34	42.0 (25.0-61.0)	100 (29.0-100)
	Age Group	Adults>18 years (1)	44	100 (81.0-100)	54 (33-73)
		Children (1)	1200	79 (66.0-89.0)	90 (88.0-92.0)
		Adults & children (1)	34	42 (25.0-61.0)	100 (29.0-100)
MOLECULAR TESTS					
LAMP: Overall accuracy (4 studies)			**(N=421)**	**99.9 (8.9-99.9)**	**76.2 (57.0-87.0)**
	Reference standard	PCR (1)	86	100 (95.0-100)	88.0 (62.0-98.0)
		Single microscopy (3)	335	99.7 (4.1-99.9)	72.3 (49.5-87.5)
	Prevalence setting	Endemic (3)	327	99.6 (20.9-99.9)	67.2 (47.4-82.3)
		Non endemic (1)	94	100 (81.0-100)	89.0 (80.0-95.0)
	Age group	Adults& children (3)	249	100	81.8 (64.3-91.9
PARASITOLOGICAL TESTS					
Mobile microscopy: Overall accuracy (4 studies)			**(N=561)**	**68.4 (54.9-73.4)**	**98.1 (97.5-99.2)**
	Device type	Portable microscope (2) Cellscope (2)	286 275	76.5 (68.2-82.3) 59 (47.3-73.5)	98.5 (97.5-99.2) 99.5 (97.5-99.2)

4.5.5.1 Reference standard:

The subgroup analysis in Table 12 reveals that when index tests are evaluated against a higher quality reference standard such as PCR, LCA or composite tests, the specificity results improve for ICT-RDT (62.5% vs 42.7%), IHA (100% vs 90,8%) and LAMP (88.0% vs 72.3%), although their sensitivity results do not change much. For POC-CCA, reference tests do not influence the accuracy results.

4.5.5.2 Prevalence setting

The co-variate prevalence setting influences diagnostic accuracy. It seems that a setting in Europe where migrants and travellers are suspected of schistosomiasis, and then screened using serological tests such as IHA or ICT-RDT gives improved specificity results compared to endemic settings in Africa where exposed populations are screened. This discrepancy is seen in IHA (99.3% vs 76.1%), ICT-RDT (73.4% vs 48.0%) and LAMP (89.0% vs 67.2%) and is a reason for heterogeneity of results for these tests. The UCP-LFCAA is far better suited to endemic settings and performs much higher when excluding the non-endemic study on acutely infected travellers (sensitivity 97.1% vs 42.0%).

4.5.5.3 Age group

The age group category is important for serological tests, as children with new infections will have more accurate results than adults who have had multiple infections and treatment. Serological tests detect antibodies to *Schistosoma* which stay positive lifelong, even when there is no active infection. For ICT-RDT, tests in children have a higher specificity than in adults (80% vs 59.2%). However, the opposite was true for LAMP and POC-CCA, where a combined population of all ages have a better specificity than school aged children (81.8% vs 52%) and (43.5% vs 66.8%) respectively.

4.5.5.4 Mobile microscope type

The two types of mobile and portable microscope also affect the overall accuracy for the category mobile microscope, where the Cellscope has much poorer sensitivity compared to the portable microscopes reviewed (sensitivity 59% vs 76.5%). All microscopy studies had the same age-group, reference standard and prevalence setting.

CHAPTER 5: DISCUSSION

The aim of this study was to assess the diagnostic performance of rapid and point-of-care diagnostic tests for the direct diagnosis of urogenital schistosomiasis (UGS) compared to laboratory-based reference tests in individuals from Africa. There is a dearth of effective direct diagnostic tools available for areas that are endemic for *S. haematobium*, such as southern Africa (38)(9). Microscopy of filtered urine remains the current gold standard for detecting schistosome infections but has major disadvantages and low sensitivity in chronic infections and low prevalence areas, preventing early detection and treatment (6).

Urogenital schistosomiasis remains an important NTD as it is the most prevalent of the NTD's affecting several million people in Africa and globally resulting in severe morbidity (2). In countries like South Africa where there is a high prevalence of HIV, the risk of contracting HIV is quadrupled among individuals suffering from chronic untreated FGS(7). There is an urgent need to improve the diagnosis and treat schistosomiasis when implementing control programs in schistosome-endemic areas. To our knowledge, this is the first systematic review to collate the available evidence on accuracy measures of rapid field-based diagnostic tests that use various methods (serological, molecular, antigenic, mobile microscopy) for the detection of *S. haematobium* specifically, in samples of serum and urine.

The findings of this review indicate that new highly sensitive and specific diagnostic tools are available for low-resource areas, particularly field-based molecular tests such as RPA and LAMP. The diagnostic gap is for an accurate field-based test that can diagnose active UGS in its acute and chronic form even in low infection intensity settings, within 2 hours of sample collection(23).
The UCP-LFCAA assay, although still in development and not commercially available was also found to have a high diagnostic accuracy for active infections even in chronic or low intensity infections.

Ten index tests were found to fit the criteria of rapid or POC tests with accuracy in low resource settings with limited laboratory equipment. Amongst these, three tests could be considered as highly accurate with a good trade-off between sensitivity and specificity. These are the RPA (sensitivity= 94.1%, specificity= 96.6%), LAMP (sensitivity=99.9%, specificity= 76.2%) and UCP-LFCAA (sensitivity=87.4, specificity=86.4). Overall, RPA is the most suitable test for

prevalence estimation in both high and low endemic settings, as well as for estimation of elimination of transmission of *S. haematobium*. LAMP and UCP-LFCAA are highly sensitive tests and can be used for screening and mapping.

Three tests (SmCTF, ICT-RDT and RDT-Sh) were found to have a high sensitivity but lacked specificity. This means that these tests are highly accurate with few false positives, yet they are assigned a low specificity due to their comparable sensitivity being superior to a poor reference standard of single microscopy. Further research could provide answers for their use as rapid tests in endemic areas.

Of the 458 studies identified in the initial identification of studies, only 33 diagnostic accuracy studies met the inclusion criteria of diagnosing urogenital schistosomiasis directly, and rapidly within 2 hours of sample collection. Most of the excluded studies were accuracy studies for the indirect diagnosis of *S. haematobium*, specifically urine reagent strips for haematuria. While urine strips are useful screening tools in endemic areas for mapping and field-based control, they cannot diagnose chronic infections and cannot be used for transmission interruption surveillance(44). Microhaematuria remains a highly useful mass mapping tool in high infection-intensity endemic areas for school-aged children(48), and is recommended by WHO as a mapping tool for these areas. However, after the initial mapping, any control program implemented is disadvantaged without an improved diagnostic tool for prevalence estimates after MDA, and in low transmission areas, for which urine reagent strips are not suitable.

Direct tests can be considered more accurate than indirect tests for screening and control program but also have limitations as many direct tests are based on immunological markers and require serum. The IHA, ICT-RDT, DDIA and SmCTF all require a serum sample, which requires a trained phlebotomist to draw blood and centrifuging equipment to separate serum from whole blood. The POC-CCA is a direct test that uses urine but has been found to be ineffective in low prevalence endemic areas for the diagnosis of *S. haematobium* (48). Our study confirms these poor accuracy results for POC-CCA (sensitivity 48.9%, specificity 77.3%). The only three direct tests in this review that require urine are the UCP-LFCAA, LAMP and RPA, making these tests very viable for field-based collection of samples.

Research is ongoing currently for accurate tests that can be considered a urine microscopy replacement, therefore among those studies excluded from this review were proof of concept studies with accuracy results from in-vitro and egg spiked urine tests. The LAMP assay has various methods of in-field DNA extraction methods whose accuracy is still being resolved in studies(83). Case-control studies for both LAMP and RPA were included in this review as samples were taken from the same population. This may have resulted in sampling bias for these index tests.

The UCP-LFCAA, and the RPA and LAMP assays are still in development but show great promise for use as a test-and-treat tools in low resource areas with field laboratories. Both RPA and LAMP are molecular tests employing field-based methods for *Schistosoma* DNA detection and can be considered rapid and close-to -patient, not POC as basic portable laboratory equipment is still necessary. More in-depth research is required to determine the full scope of accuracy in low resource areas for the UCP-LFCAA, and the RPA and LAMP assays.

5.1 Comparisons with other DTA reviews

A similar systematic review and meta-analysis published by Vaillant *et al* (2021) assessed the accuracy of diagnostic tests for both species of *Schistosoma* in low prevalence areas(84). Vaillant's results are comparable to ours for the POC-CCA test for sensitivity and specificity (51,43% and 71,13%) compared to this review (48.9% and 77.3%), but they had lower results for the LAMP test (77.06% and 63.50) versus our review (99.9% and 76.2%). The CAA test (70.93% and 78.57%) in their review was an ELISA based on CAA, and not the same test as the UCP-LFCAA test in our review (87.4% and 86.4%) which is far more rapid with less equipment needed(85) (51).

Our current study found higher accuracy results for the SmCTF-RDT compared to a narrative review by Hinz *et al* (2017). The results in their review for SmCTF-RDT are for both *Schistosoma* species and have lower results (sensitivity 67%, specificity 33%) vs our review (sensitivity 98%, specificity 33%), which highlights how this test could be further studied for *S. haematobium* predominant areas.

5.2 Comparisons of diagnostic accuracy of eligible index tests

The current systematic review was able to perform an indirect comparison between the ten tests eligible for the study. Comparisons were made visually on a summary ROC plot and revealed that the ROC curve for the RPA test has the highest overall diagnostic accuracy, followed by the LAMP

assay. The relative diagnostic odds ratio (RDOR) is the highest point on each curve on the ROC plot is a valid reflection of the difference in accuracy of the various index tests. Through meta-analysis LAMP had the highest DOR (DOR=5532), followed by RPA. Comparison of the absolute difference in sensitivity and specificity summary measures between RPA and LAMP reveal however, that these two tests have no statistically significant differences.

The results reveal that both mobile microscope and IHA are not suitable for mapping or estimation of infection in an area. Furthermore, 4 tests had inaccurate results displaying a higher false positive rate than those infected due to using single microscopy as a poor-quality reference standard. These are POC-CCA, ICT-RDT, SmCTF and UCP-LFCAA, and improved studies using higher quality reference tests may shed light on the performance of these tests.

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5.3 Strengths and limitations of the review

5.3.1 Strengths

Relevancy: This review evaluates tests currently in use for the diagnosis of *S. haematobium*, as well as new tests that are in the process of commercialization. This review is therefore relevant to current clinical practice.

Reference standards: The choice of a wide range of laboratory-based reference standards can be considered a strength, as previous reviews chose microscopy as a reference standard, thereby compromising the accuracy of their results. However, this makes the results of this review highly heterogenous, and therefore future test performance studies should use higher quality reference tests to improve and verify the accuracy of the test performance results of this review.

5.3.2 Limitations:

Unpublished studies are currently underway for the newly developed SCH CAA RDT, which is in a late stage of development(86). Field evaluations by the diagnostic test advocacy group FIND (www.finddx.org) are underway in Kenya and the Philippines with 1556 individuals enrolled to determine the feasibility of this test, however nothing is published as yet, and therefore not included in this review. (87).

Other unpublished studies by the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) developed in 2008 and, funded by the Bill & Melinda Gates foundation, have put focus into improved diagnostic tools for *S. haematobium* with focus on the CAA antigen. A large prospective observational study across 3 countries in Africa was registered in 2018 for the

measurement of the performance of CAA as a diagnostic tool(88). This trial, called the FreeBILy project aims to investigate the use of the CAA antigen in rapid tests for the diagnosis of *Schistosoma* infection, and published results are not yet available, but were expected in 2022. Therefore, the scope of this review in terms of test accuracy comparisons may be improved if these results had been published.

Inconsistent reporting and heterogeneity: Our investigations into heterogeneity between studies was limited due to inconsistent reporting of some variables. Infection intensity with regards to individual infections of each person differs, depending on their repeated exposure to infected water. A higher intensity results in higher egg numbers released compared to a lower intensity. Although some studies reported egg intensity, we did not include this variable in the investigations into heterogeneity, which is a limitation. Our study also did not investigate whether an infection was acute or chronic for populations in Africa, or as migrants or travellers in Europe. The timing of attaining an infection before being tested is an important variable and will give differing test results in different populations. Most studies did not report whether an infection was chronic, but it can be inferred that migrant populations have more long-standing infections. One study by Hoekstra reported accuracy results for the UCP-LFCAA on a population of returning travellers with acute infections, but for all other studies in this review the chronicity of an infection was not reported. These inconsistencies may be reasons for heterogeneity in this review.

Low numbers of studies: Analyses could not be performed adequately due to sparse data available, where 4 studies or less were available for 8 of the 10 index tests analysed. Therefore, the certainty of the evidence available has low accuracy for the majority of the tests evaluated in this review. Only POC-CCA and ICT-RDT contained more than 4 studies to be meta-analysed, where 8 index tests had 4 studies or less with a high heterogeneity between studies.

5.4 Applicability of the findings to the review question

The findings of this review had some concern in terms of applicability to the review question, with regard to index test and reference used as comparator. We were able to obtain summary estimates for 10 eligible direct POC or rapid tests for diagnosis of urogenital schistosomiasis, as well as compare their accuracy. The population were all individuals who were exposed to *S. haematobium* in African countries and tested or screened either in endemic field settings as populations living in Africa, or non-endemic settings as travellers or migrants from African countries. Potential sources of heterogeneity regarding age-group, reference test and prevalence were assessed, but

incompletely reported variables regarding infection intensity, chronicity, and study design may limit the applicability of these findings. Objective assessments of the strength of evidence of each study were assessed using the QUADAS -2, where risk of bias regarding patient selection, reference test, index test, setting and flow/timing were assessed. Not all index tests distinguished an infection of *S. haematobium* from a healthy non-infected individual as 6 out of 10 tests could not specify which genus of S*chistosoma* was detected. In populations with mixed *S. haematobium* and *S. mansoni*, the results reported for this review were referenced by the urine microscopy egg detection for patients with *S. haematobium* infection only. Patients with co-infection with both species were excluded. However, for two tests, Sm-CTF and ICT-RDT, the results obtained were mixed genus infection, as the tests were employed rather as a general screening for all *Schistosoma* species. This may cause limitation to the applicability of the findings; however, efforts were made to ensure only *S. haematobium* populations were included in the results. The index tests identified are all direct tests, detecting *Schistosoma* antigen, antibody or DNA from serum or urine, however only 4 tests were specific for *S. haematobium*. These are LAMP and RPA (detecting the Dra1 genomic sequence from *S. haematobium*), mobile microscope (detecting *S. haematobium* eggs), and Sh-RDT (detecting antigen on the *S. haematobium* egg). The remainder of the tests reviewed are not specific for *S. haematobium* and can be employed for mapping or screening purposes in settings of mixed species infections.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

New innovations of rapid and field- based tests for *S. haematobium* show great promise for the successful implementation of control strategies in resource limited settings. Most tests eligible for this review are still not fully POC and require field- based laboratories for serum attainment(18,65). Many tests are developed with innovative field- based kits, and table-top portable equipment and are included under the definition as rapid tests if results were available within 2 hours. Therefore, the tests included in this review are clinically relevant for testing at or near the patient's side. More research is needed to verify the accuracy results for the molecular tests RPA and LAMP that showed the highest diagnostic accuracy results, the ICT-RDT which can be considered the most accurate screening test, as well as the UCP-LFCAA which shows the most promise for determining an active infection.

Conclusive results cannot be drawn from this review and further research is needed to verify accuracy for all 9 of the 10 tests found eligible. The only test for which accuracy results can be considered plausible is the POC-CCA, where 11 studies were analysed for accuracy in *S. haematobium* dominant areas, with low heterogeneity between the studies. The results are comparable to other reviews and studies, concluding that the POC-CCA is not suitable to test for *S. haematobium*.

The most promising studies use improved reference standards such as PCR, double/triple microscopy, or composite tests. Microscopy is an imperfect reference standard which introduces bias for tests that are more sensitive than microscopy. Five of the ten tests eligible for this review were found to have a high false positive rate, which indicates that the test detects more infections than the reference test. Therefore, specificity results for LAMP, SmCTF, ICT-RDT and UCP-LFCAA are possibly biased, and need to be verified in future accuracy studies that use a higher quality reference test.

6.2 Recommendations

6.2.1 Implications for practice

Diagnostic gaps have been recognized for areas where *S. haematobium* is the predominant species. This review has identified possible tests that have the potential to replace or complement traditional laboratory- based tests, particularly in low resource areas where patients have limited access to

health facilities. The rapidity of a test, and its result at the patient side is of great value to patients who otherwise may not return readily to receive their results, especially in areas with few or poorly equipped health facilities. We have identified two molecular tests, RPA and LAMP, and a rapid antigen test UCP-LFCAA that can be implemented to diagnose acute, sub-acute and chronic stages of urogenital schistosomiasis in field laboratories in poor resource areas with some training of laboratory staff, and minimal equipment. We have also identified ICT-RDT and possibly SmCTF-RDT as highly sensitive screening tests for all species of *Schistosoma*, and effective for mapping, and prevalence screening with rapid near patient results. An effective control program should implement testing of populations after mass drug administration, to determine whether transmission interruption has occurred in a particular area. We have identified RPA as a highly accurate rapid test which functions optimally in low prevalence settings of 2.5% infection prevalence.

We would recommend that more qualitative studies be implemented seeking the perspectives of the community about whether mass drug administration is suitable and accepted in communities. A test and treat strategy may be of great value for improving the support of parents, teachers and community leaders in gaining the consent of the recommended 75% of a community for mass drug administration strategies.

6.2.2 Implications for Research

Research into the development of true POC tests conducted near or at the patient's side are urgently needed for the diagnosis of urogenital schistosomiasis, particularly for populations with concomitant high incidence of HIV. The true impact of neglecting the treatment of urogenital schistosomiasis in populations with high HIV incidence and acquisition has not been fully assessed, and a more complete understanding of the association of HIV and FGS will advance the research agenda for diagnosis and uptake of control programs in areas of high prevalence of urogenital schistosomiasis.

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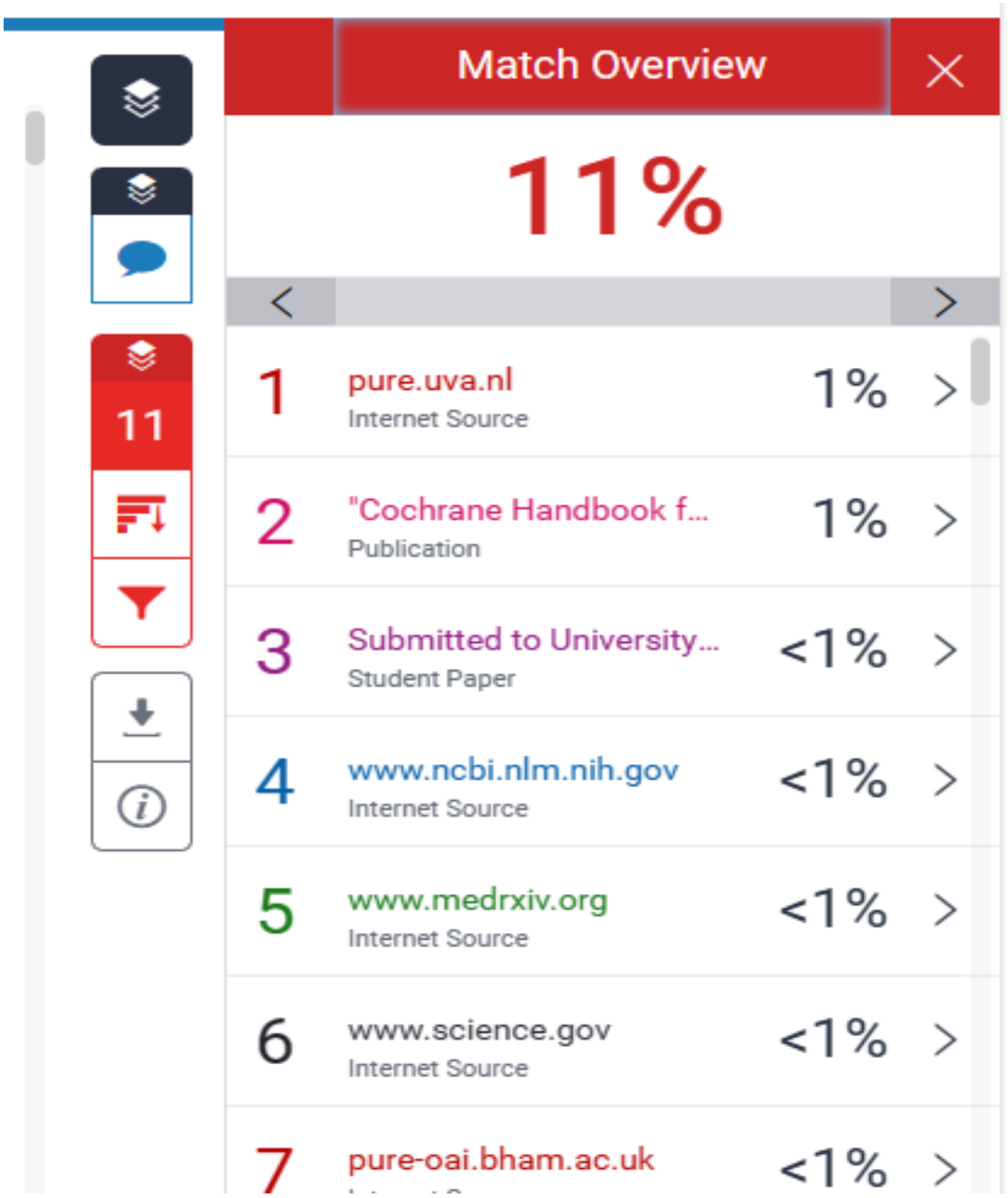
APPENDICES

Appendix A: Plagiarism/Turn-it-in report



Appendix B: Ethics waiver certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Innovation)

TO: Ms E Draxl
School: Public Health
Department:
Division: Epidemiology and Biostatistics
Medical School
University
E-mail: ericavanmey@gmail.com

CC: Supervisor: Dr N Ndlovu <Zodwa.Ndlovu@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 25/05/2023

REF: *R14/49*

PROTOCOL NO: W-CBP-230525-01 (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

PROJECT TITLE: *The diagnostic accuracy of point of care tests for Schistosomiasis haematobium - a systematic review*

Please find attached the Ethics Waiver Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/ClearScanWaiver.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Innovation)

25/05/2023

Ref: W-CBP-230525-01

TO WHOM IT MAY CONCERN

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical)

Investigator: Ms E Draxl
Student No. (if appropriate): 95 0012 1E
Staff No. (if appropriate):

Supervisor: Dr N Ndlovu

School: Public Health
Department:
Epidemiology and Biostatistics
Medical School
University

Project title: *The diagnostic accuracy of point of care tests for Schistosomiasis haematobium - a systematic review*

Reason: Review of information in the public domain
No human participants will be involved in the study

CB Penny

Dr CB Penny
Chairperson: Human Research Ethics Committee (Medical)

Research Office Secretariat:
Third Floor, Phillip Tobias Building, corner of St Andrews and York Roads, Parktown, Johannesburg 2193
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https://www.wits.ac.za/research/researcher-support/research-ethics/ethics-committees/

Appendix C: PECO

PECO for Systematic review of diagnostic accuracy of point of care tests for *S. haematobium*

Population: Participants in diagnostic accuracy studies, of any age in African countries who are exposed to *Schistosoma haematobium* sub-grouped in:

- endemic areas with high intensity infections
- and non-endemic areas, post MDA populations or low intensity infections

Exposure: Index test: The index test for this systematic review is any rapid or POC test that is sensitive to distinguish an infection of *S. haematobium* from a healthy non-infected individual. Details about sample preparation techniques and in-field equipment needed should be stated, as well as thresholds used for test positivity and factors relevant to test interpretation. Possible tests/test protocols will to be ascertained by perusing the studies in the review, however the test should be a Point of Care or rapid test either commercially available or in the process of development.

Comparison: The Reference Test: The gold standard reference test for the diagnosis of urogenital schistosomiasis is microscopy of filtered urine using a light microscope at 40x magnification if the schistosomiasis prevalence of >10%. To prevent bias, for prevalence of <10% an adapted reference standard that states appropriate accuracy and sensitivity in the absence of a 'gold standard' may be used as a comparator to the index test such as PCR.

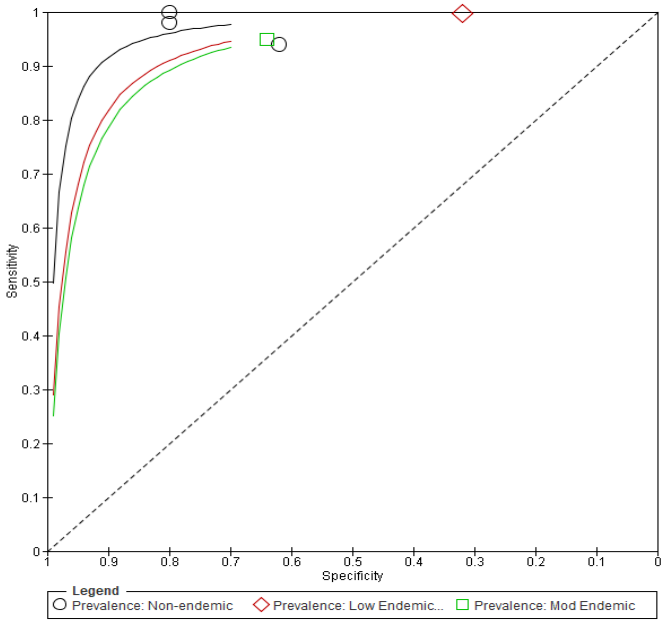
-Diagnosis of urogenital schistosomiasis: 1. Urine filtration and microscopy 2. PCR urine

Outcome: The primary outcome of interest for this systematic review is the data required to populate 2 x 2 contingency tables. A contingency table can describe at what point the diagnostic threshold is in the relationship between the index test versus the reference standard. The diagnostic threshold is the point at which results are classified as positive or negative. The table includes the number of true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN).

Appendix D: Subgroup Analyses

1. Exploratory Analysis of Subgroups for ICT-RDT using Summary ROC plots.

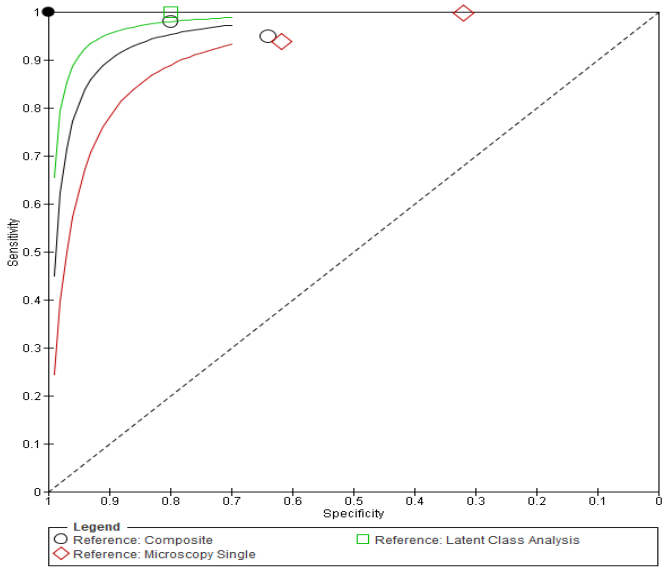
1.1.Prevalence setting: Low, Moderate, Non-endemic



The black line represents the 3 studies performed in Europe in a non-endemic area on migrants and travellers. The green line is the study by Houmsou representing an endemic area with a moderate prevalence, and the red line represents the study by Mudenda in a low prevalence. The tests performed in the non-endemic area clearly show superiority to endemic areas.

Summary ROC plot of ICT-RDT for prevalence setting subgroups

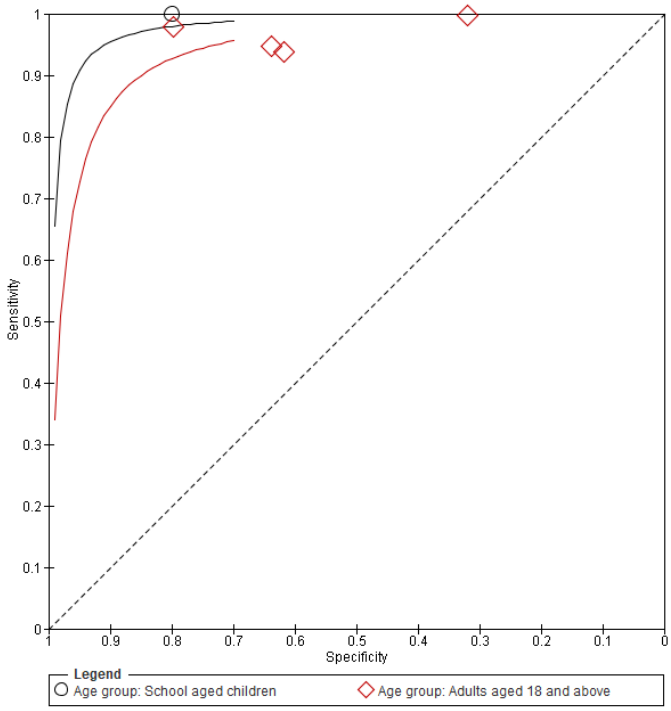
1.2. Reference test: Composite, Single, Latent Class Analysis



Both the latent class analysis (green line) and the composite reference (black line) show superior diagnostic performance compared to the single microscopy (red line). The ICT-RDT has a high sensitivity, and its specificity is therefore inaccurately portrayed by the usual gold standard using single microscopy of filtered urine.

Summary ROC curve of ICT-RDT for reference test subgroups

1.3.Age group: School aged children, Adults aged 18 and above

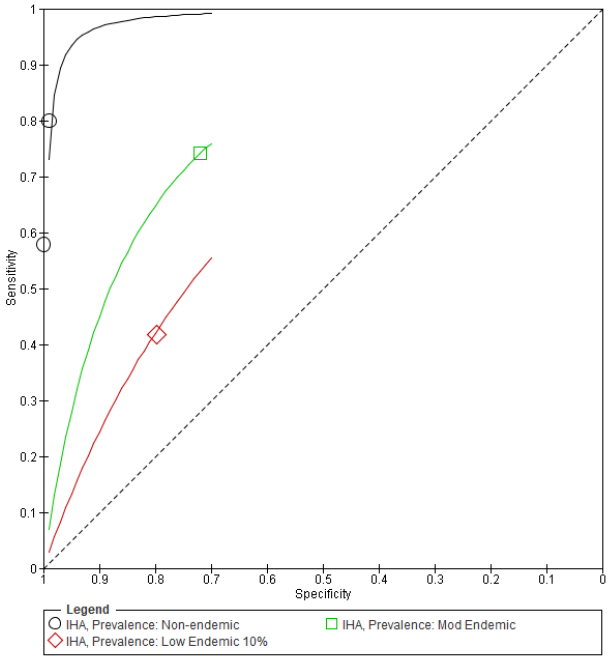


The ICT-RDT test performs better for school-aged children represented by the black line, compared to adults aged 18 and above (red line), although both are high performance.

Summary ROC curve of ICT-RDT for age-group subgroups

2.Exploratory subgroup analysis for IHA using Summary ROC plots.

2.1.Prevalence setting: Non-endemic, Low endemic, Moderate endemic

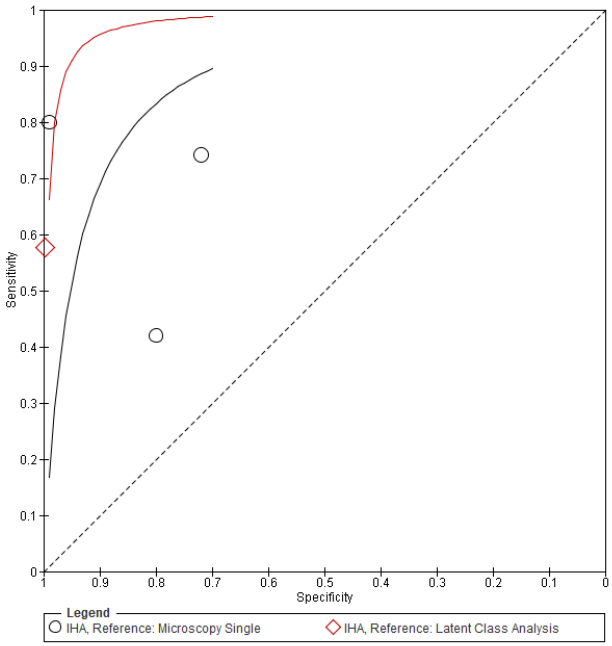


The IHA test is clearly not suited for low prevalence settings

such as that done by Yameny (red line) and performs better in a higher prevalence (green line). The black line represents studies done in non-endemic areas using improved reference standards and therefore show better diagnostic performance

Summary ROC plot for IHA based on prevalence setting

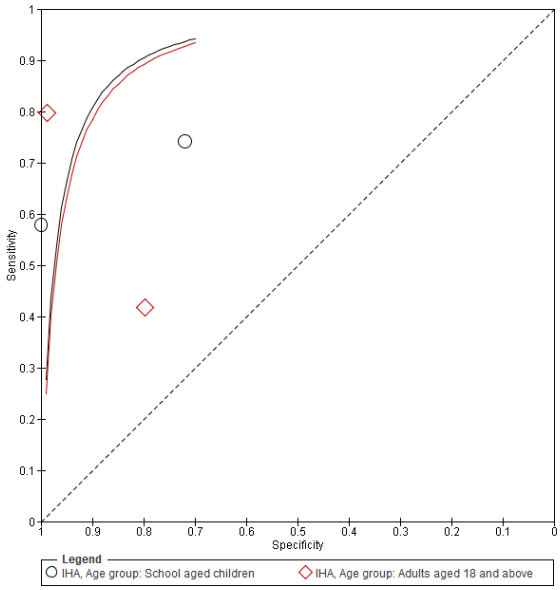
2.2 Reference Standard



Since 3 studies were case control studies using a perfect reference of chosen positive *S. haematobium* patients, there are only 2 studies to compare. The single microscopy used as a reference (black line) shows far lower performance results compared to the study using latent class analysis as a reference (red line). Therefore, bias is introduced when comparing tests using different reference standards with varied diagnostic performance levels.

Summary ROC plot for IHA based on reference standard test

2.3 Age group

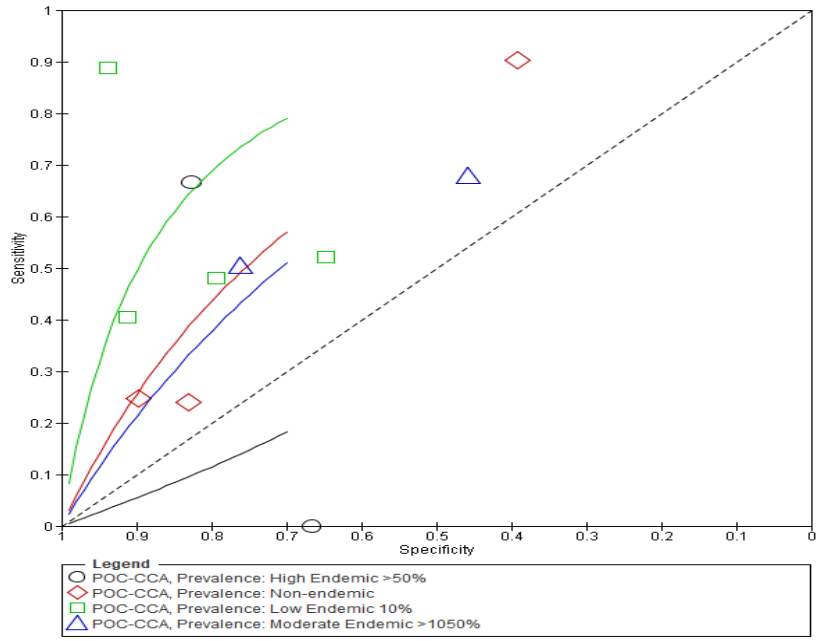


The IHA test performed better in school aged children (black line) compared to adults (red line). However, the differences are very small based on the close lines.

Summary ROC plot for IHA based on age -group.

3.Exploratory Subgroup analysis for POC-CCA

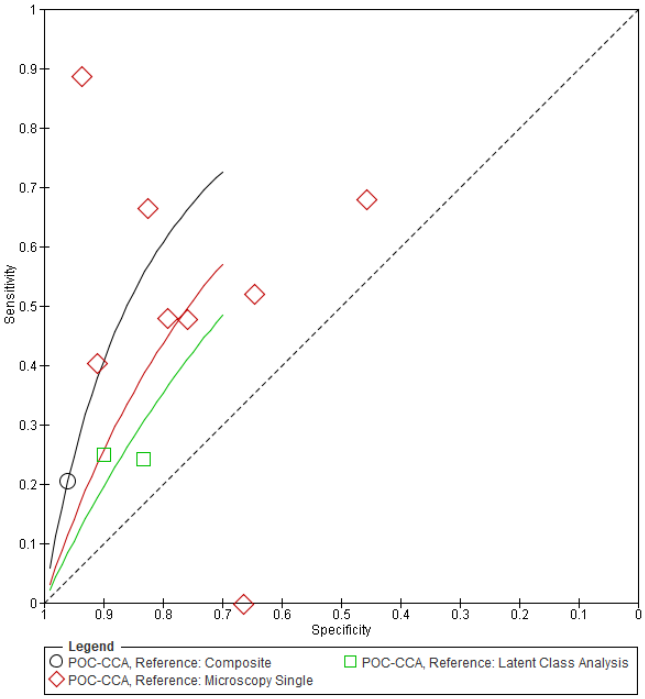
3.1 Prevalance setting



The study by Stothard (black line) was performed in an endemic region with a high prevalence, and yet he did not find that the test could diagnose a positive test at all. The TP rate was 0 in his study. The studies that were performed in a low prevalence endemic area had a better sensitivity than other settings.

Summary ROC plot of POC-CCA for subgroup analysis of prevalence setting

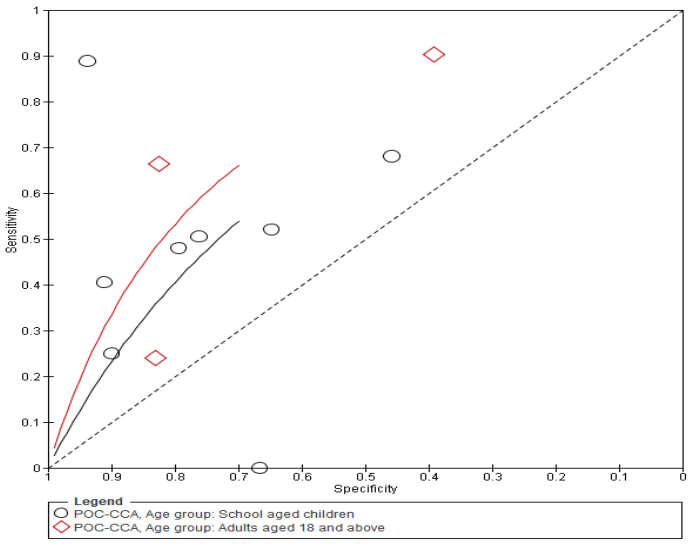
3.2 Reference standard



The study that used a composite reference test (black line) had better sensitivity results than studies that used single microscopy and LCA. LCA as a reference standard had the lowest accuracy, whereas single microscopy had improved accuracy.

Summary ROC plot of POC-CCA for subgroup analysis of reference test

3.3 Age group

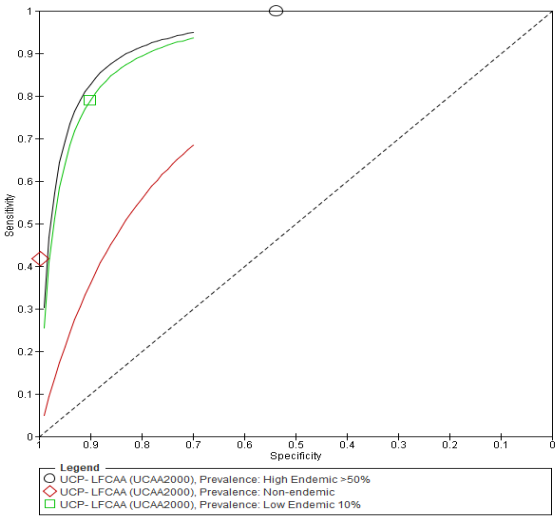


Adults aged 18 and above (the non-endemic studies) had better diagnostic accuracy results compared to children for the POC-CCA. Both groups still gave low accuracy results

Summary ROC plot of POC-CCA for subgroup analysis of age-group

4.Exploratory subgroup analysis for UCP-LFCAA test using Summary ROC plots

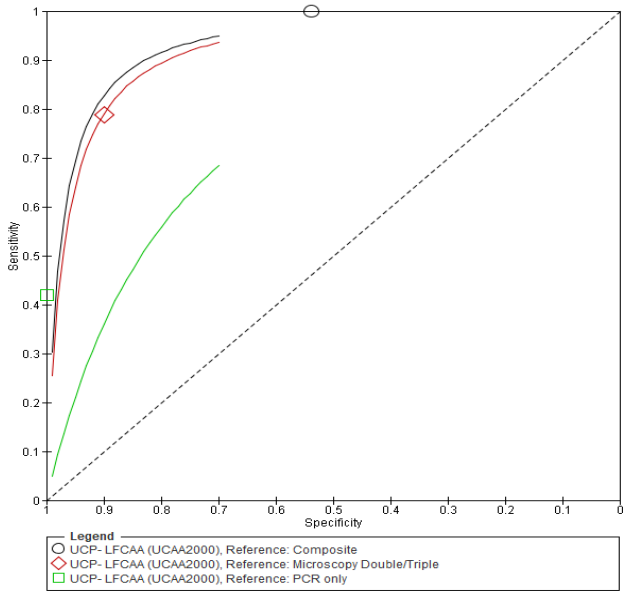
4.1 Prevalence setting



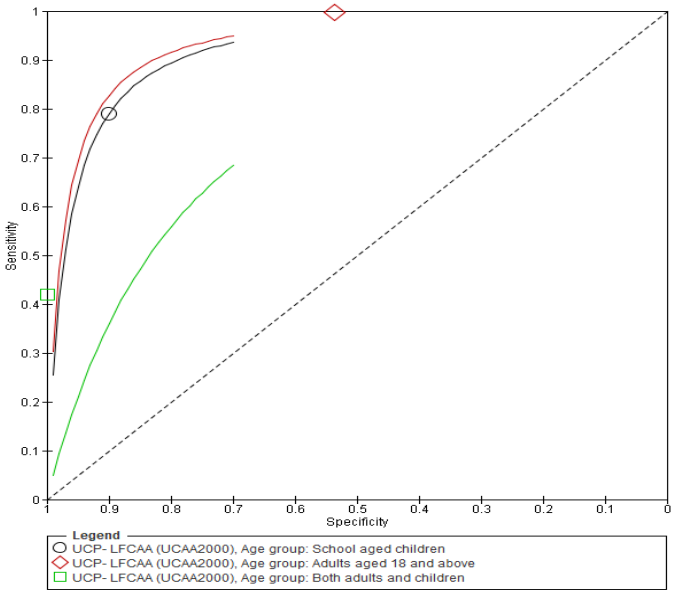
Endemic settings both high and low are well suited for the UCP-CAA test, which performs poorly for the study performed in non-endemic settings for screening of acute stage schistosomiasis in travellers

Summary ROC plot of POC-CCA for subgroup analysis of age-group

4.2 Reference test

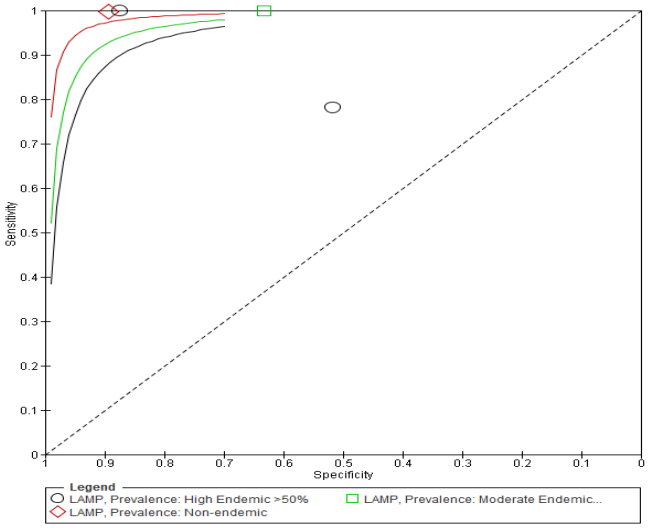


4.3 Age group



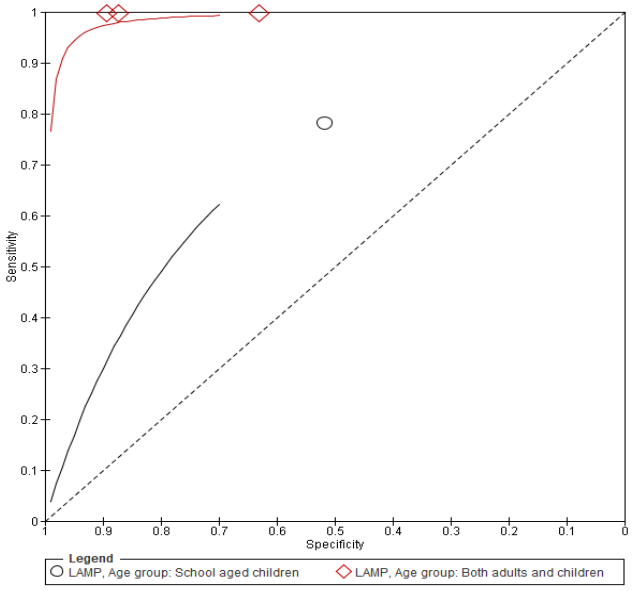
5.Summary ROC Plots for LAMP subgroups

The summary curves below represent the subgroup results for prevalence, reference test, and age-group. The subgroup ROC curves may visually give reasons for heterogeneity. The non-endemic study reveals higher accuracy than the endemic studies. Taking the study design into consideration may also infer higher results, as the non-endemic study was a case control with a perfect reference. There is little heterogeneity for the subgroup prevalence setting, all curves are similar.

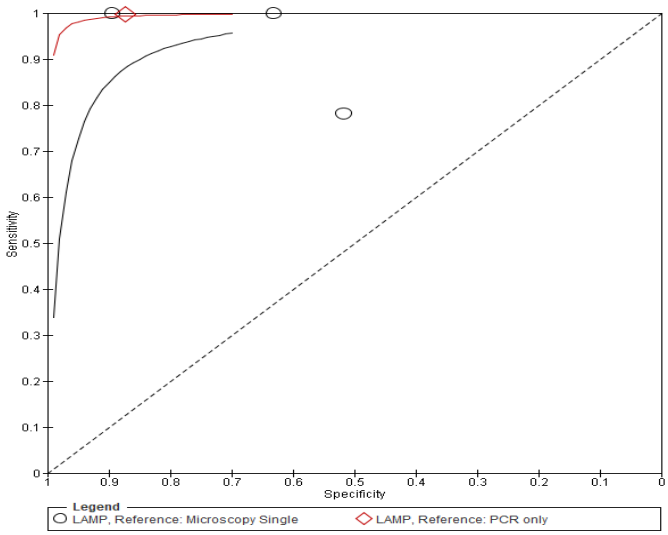


: Summary ROC plot for LAMP based on prevalence setting.

Age group subgroup analysis reveal large heterogeneity in accuracy results. School aged children reveal far lower accuracy than the LAMP assay performed in adults.



Summary ROC plot for LAMP based on Age group

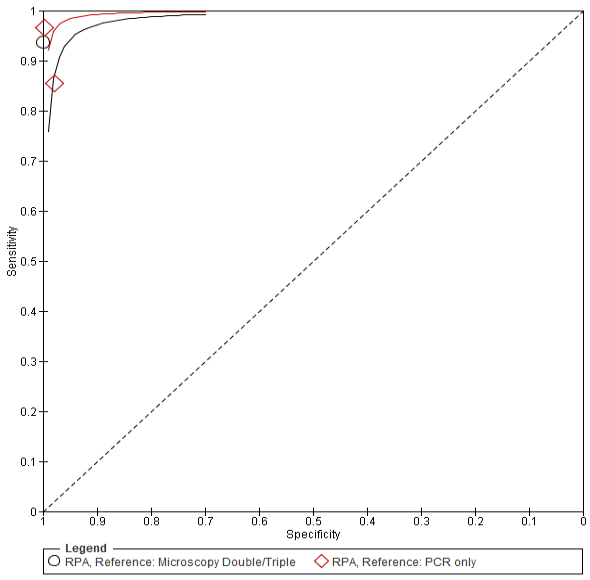


The PCR reference standard reveals higher accuracy results than using single microscopy, and therefore the subgroup analysis for reference standard reveals high heterogeneity.

Summary ROC curve for LAMP based on subgroup of reference test

6.Exploratory subgroup analysis for RPA assay using Summary ROC plots

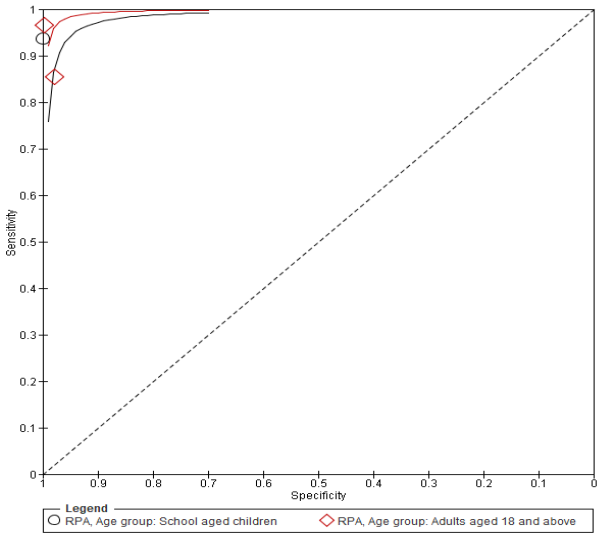
6.1 Reference test



Based on the subgroup ROC plot, the studies that used PCR as a reference performed higher than studies that used microscopy as a reference.

Summary ROC plot for RPA based on reference standard

6.2Age group



There is slight heterogeneity in the age subgroup where adults 18 and above performed better than school-aged children.

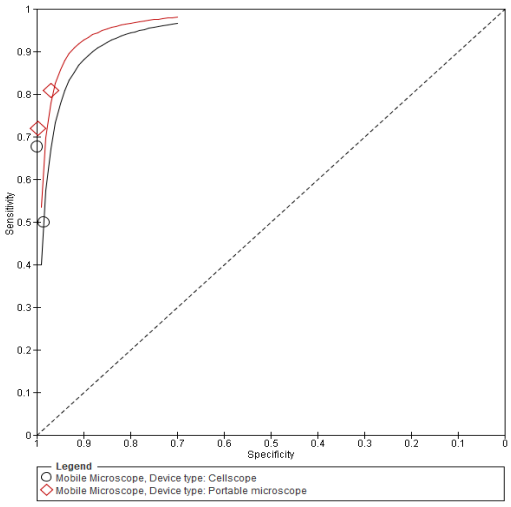
Summary ROC plot for RPA based on age group

<u>7.Exploratory subgroup analysis for mobile microscope using Summary ROC plots</u>

7.1 Device type

Subgroups of reference type, age group and prevalence are all the same or similar for all three of the mobile microscope tests, therefore heterogeneity cannot be explored using these groups.

The only source of heterogeneity for the mobile microscope test is the device type, as three different devices are analysed. The Cellscope uses the light and camera lens from the mobile phone itself, whereas the Newton NM1 and the UCLA novel microscope use their own light source and lens. The UCLA microscope combines the lens of the mobile phone camera with its own device lens. These differences may be a reason for heterogeneity in accuracy results.



Summary ROC plot for mobile microscope based on device type