Leiden University Medical Center

October 25th 2021

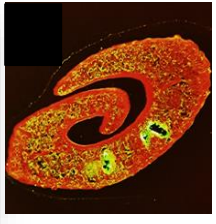
https://youtu.be/vPLway6jP_I

Leiden University Medical Center

Schistosomiasis diagnostic research at the LUMC: From discovery to implementation

Dr. Lisette van Lieshout
Leiden University Medical Center, Netherlands
Department of Parasitology




Disclosure:
Department provides the αCAA αCCA McAbs
αCCA to Rapid Medical Diagnostics (South Africa) for urine POC-CCA cassette

In memoriam Professor A.M. Deelder (1947 – 2021)

EXPERIMENTAL PARASITOLOGY 40, 189–197 (1976)

Schistosoma mansoni: Demonstration of Two Circulating Antigens in Infected Hamsters

A. M. DEELDER, H. T. M. KLAPPE, G. J. M. J. VAN DEN AARDWEG, AND E. H. E. M. VAN MEERBEKE

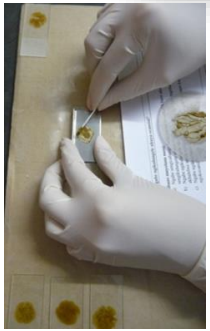
Laboratory of Parasitology, University of Leiden, Rapenburg 33, Leiden, The Netherlands

(Accepted for publication 27 October 1975)

Laboratory diagnosis of schistosomiasis

Microscopy

- Endemic regions: urine /10 mL; stool Kato-Katz
- WHO reference standard, quantify
- High specificity, but lacks sensitivity
- Simple, but operator dependent

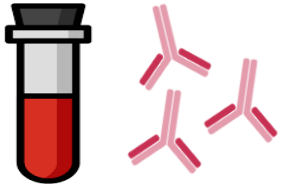


Lack of sensitivity -> repeated

Non-microscopy laboratory diagnosis of schistosomiasis – LUMC research

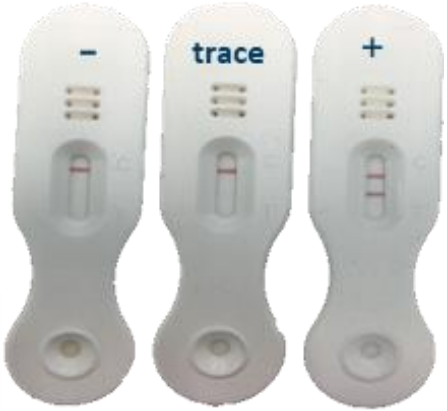
1. DNA detecting tests (NAAT)

2. Serology (Ab testing)



3. Circulating antigens

- Commercial POC-CCA
- In-house UCP-LF CAA

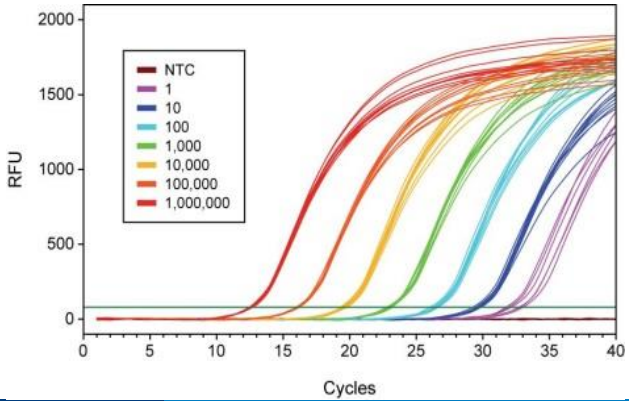


For review a.o.: Utzinger *et al.*, Clin Microbiol Infect. (2015); Hoekstra *et al.*, (2021)

DNA detection: NAAT-based diagnosis

- LUMC: DNA in excreta (faeces, urine, sperm, swaps, lavage)
- (Others: cfDNA: blood-based PCR => urine-excretion)
- Real-time PCR, semi-quantification options (Ct-value)
- Highly specific by design
- Highly sensitive (but not always 100%!) – proxy for egg excretion
- Rapidly cleared (<weeks) after treatment

(-) High-tech procedure



LUMC: ITS-2, genus (S.m./S.h.) Obeng et al., 2008
Routine diagnostics since 2007 (ISO 15189)

Participation in proficiency testing (EQAS)
Cools et al. (2020) PLosNTD

Female Genital Schistosomiasis

Need for user-friendly diagnosis

Zambia

2.5% Vaginal Swab = 15/603

3.3% Cervical Swab = 20/603

4.0% Any Swab = 24/603

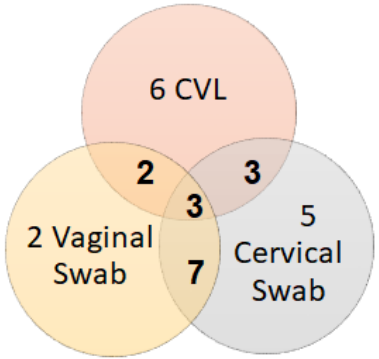
RESEARCH ARTICLE

PLOS NEGLECTED TROPICAL DISEASES

Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study

Amy S. Sturt[1]*, Emily L. Webb[2], Comfort R. Phiri[3], Tobias Mweene[3], Namakau Chola[3], Govert J. van Dam[4], Paul L. A. M. Corstjens[5], Els Wessels[6], J. Russell Stothard[7], Richard Hayes[2], Helen Ayles[1,3], Isaiah Hansingo[8], Lisette van Lieshout[4], Amaya L. Bustinduy[1]

2.7% Cervicovaginal Lavage (CVL) = 14/527



70% of those with FGS had active *Schistosoma* infection* (21/30)

*defined as positive **urine** PCR, CAA, or microscopy

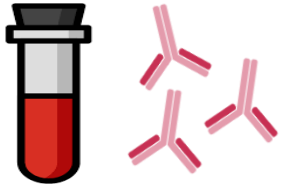
a.o.: Sturt *et al.*, PLosNTD (2020); Sturt *et al.*, (2020, 2021) Rafferty *et al.*, (2021); Bustinduy *at al.*, (review in preparation)

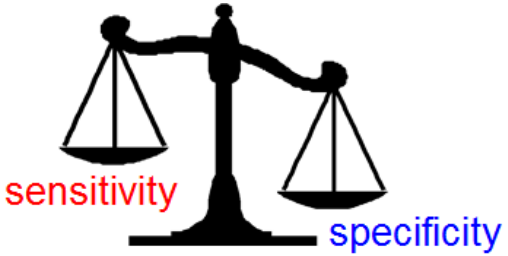
Serology: anti-*Schistosoma* antibody detection

Commercial tests/in-house tests

Specificity??

Sensitivity??





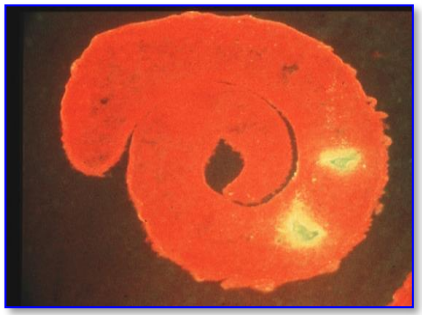
No correlation with intensity of infection/clinical symptoms

Not for evaluation of treatment

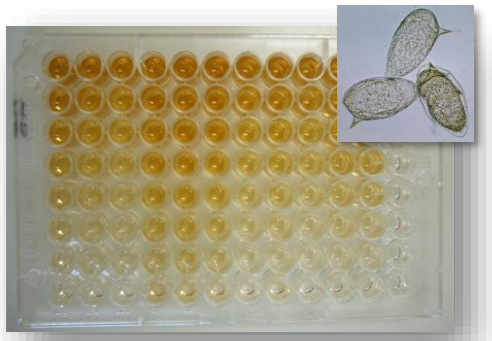
LUMC: in-house tests; ISO 15189

IFA – IgM α-AWA Nash *et al.*, 1978 AJTMH

ELISA – IgG α-eggs (SEA)



IgM - IFA:
Rossman's fixed male S.m. adult worms

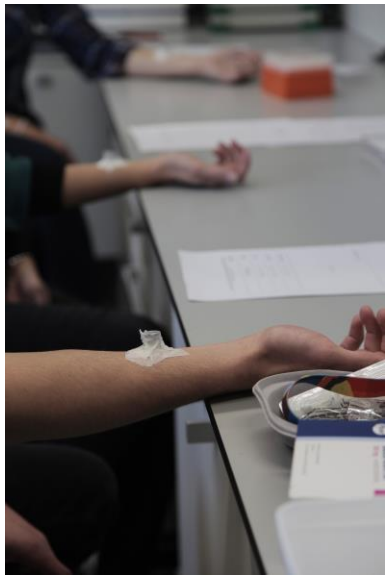


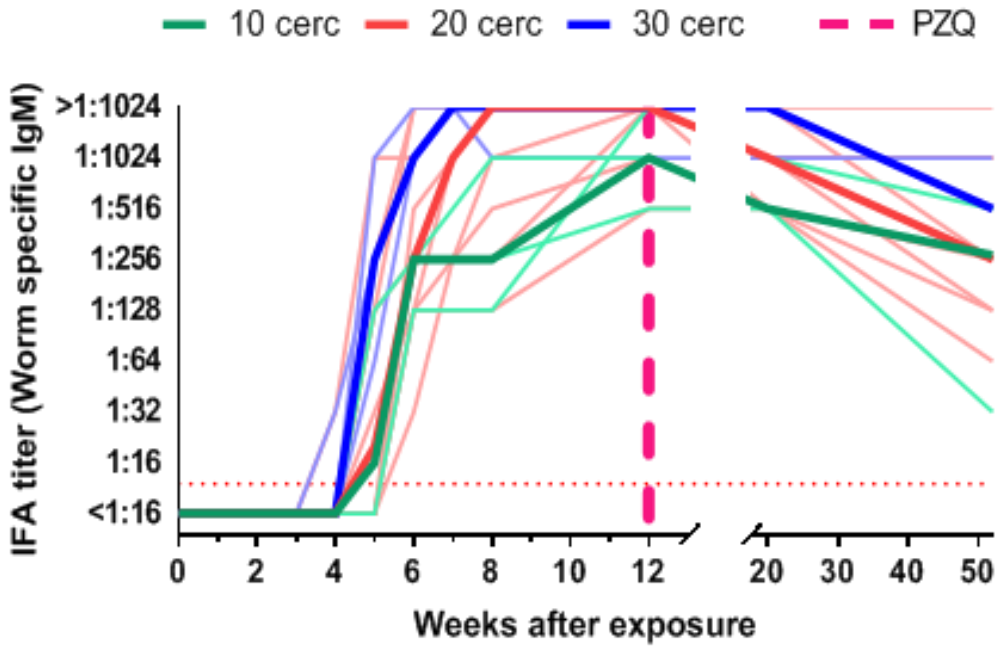
IgG - ELISA:
crude S.m. soluble egg antigen (SEA)

Controlled Human Schistosoma infection (CoHSI)
Diagnostic aspects – antibody detection

Healthy Dutch volunteers infected with 10 (n=3), 20 (n=11) or 30 (n=3) MALE *S. mansoni* cercariae

Meta Roestenberg and team





Langenberg, Roestenberg *et al.*, (2020) Nature Medicine 26(3): 326-32

Different performance of different Ab-tests

PhD Pytsje Hoekstra

Early diagnosis and follow-up of acute schistosomiasis
in a cluster (n=34) of infected (*S. haematobium* complex)
Belgian travellers (exposed in South Africa)

PZQ

	Pre-treatment		Post-treatment
	Week 4-5 post-exposure n (%)	Week 7-8 post-exposure n (%)	Week 13-14 post-exposure n (%)
Serology (Ab)			
2 commercial test (ELISA and/or IHA positive) by ITM	3/33 (6%)	12/34 (35%)	11/34 (32%)
AWA-IFA (in-house LUMC)	13/33 (39%)	23/34 (68%)	25/34 (74%)

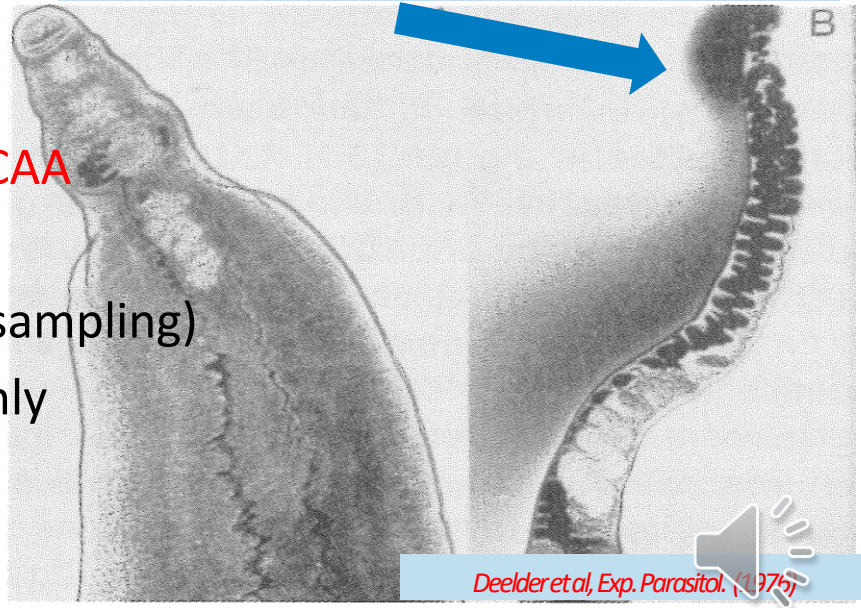
Circulating antigens
Born out of need for an alternative to microscopy

1. Detecting active infection (worm loads)
2. Highly field applicable and user friendly
3. Highly sensitive; specific
4. Case detection and for monitoring intervention

a.o.: Circulating Cathodic Antigen (CCA)
Circulating Anodic Antigen (CAA)

Circulating *Schistosoma* antigens CCA and CAA

- Adult worm derived glycans
- Serum => urine excreted (non-invasive sampling)
- Reflect parasite load, active infection only
- Cleared after PZQ

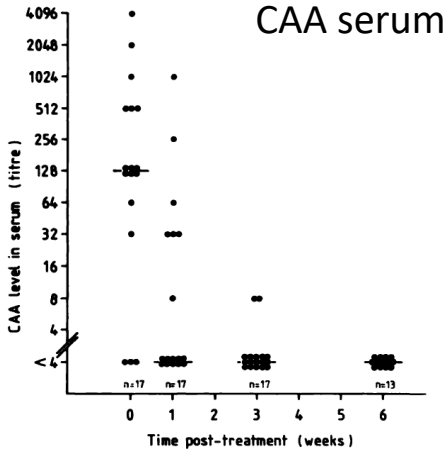


Deelder et al, Exp. Parasitol. (1976)

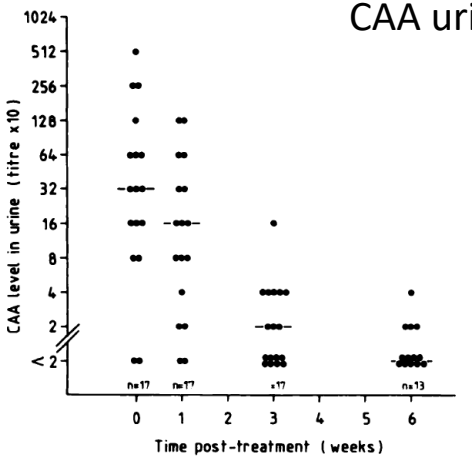
o.a. Corstjesns et al., (2014) ; Van Dam et al., (2005)

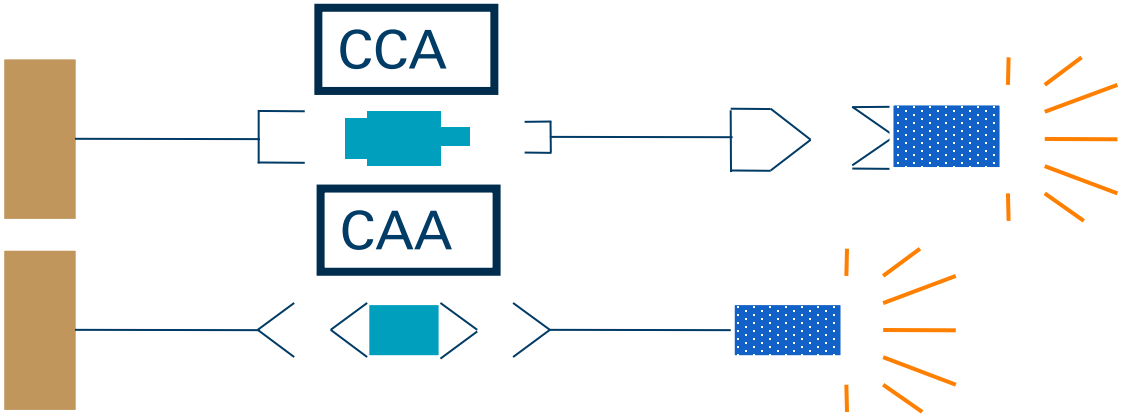
Sandwich ELISA for detection of circulating antigens

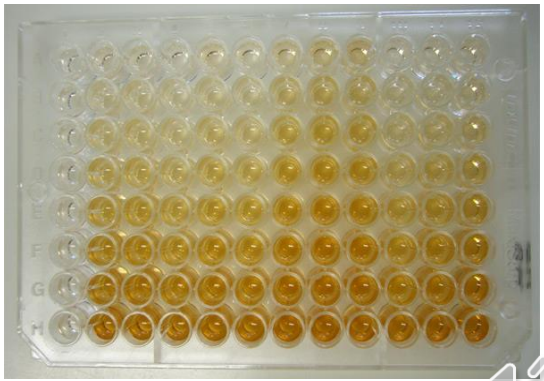
CAA serum



CAA urine



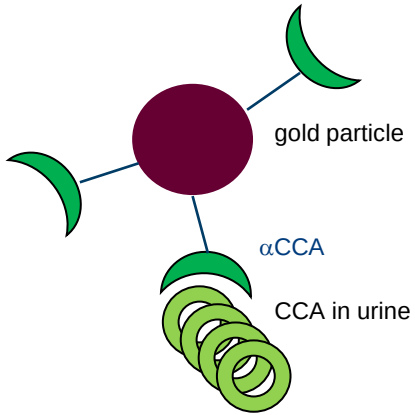




McAb - (TCA-treated) sample - McAb - enzyme substrate

Deelder *et al*, AJTMH (1989); De Jonge *et al*, TRS-TMH (1990); Van Lieshout *et al.*, AJTMH (1991)

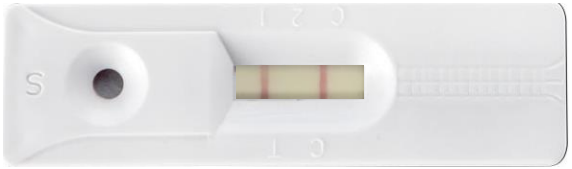
Urine POC-CCA strip assay - test principle
Aim = field applicable & user friendly

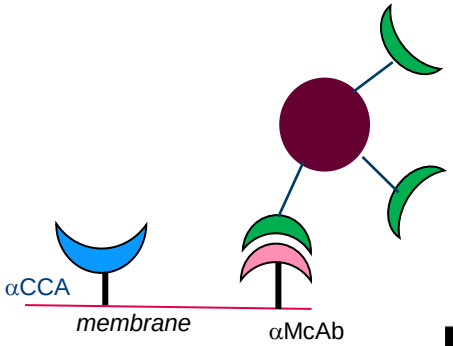


Govert van Dam and team

http://rapid-diagnostics.com/

rapidmedical diagnostics





Silveira et al PLOS NTD 2016

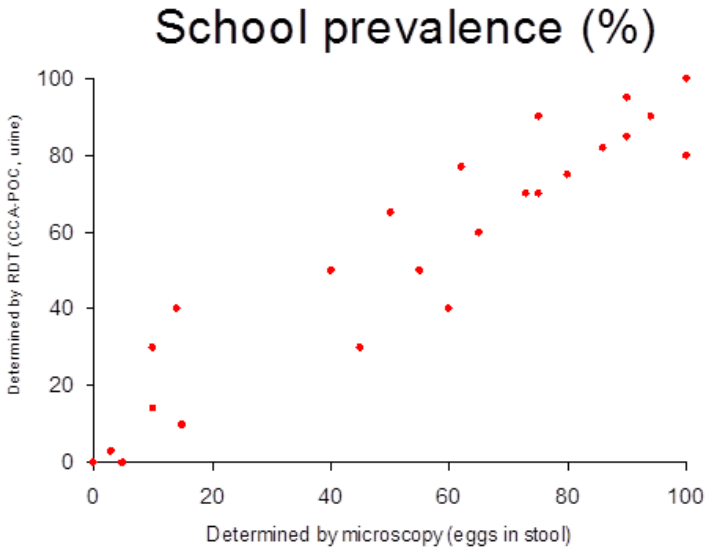
Time limitation:
Exact reading at 20 min.

Observer dependent scoring

Urine POC-CCA strip assay => population studies

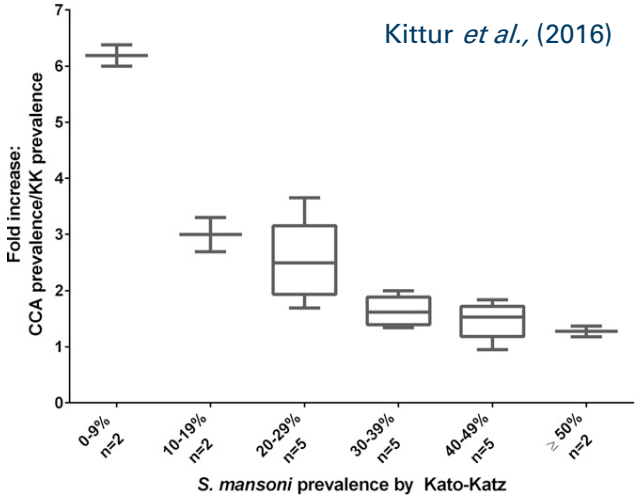
25 schools, 20-30 children per school

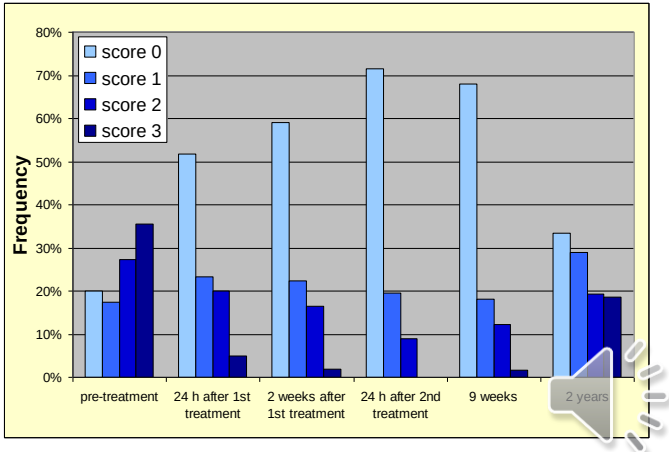
POC-CCA urine test vs stool microscopy



S. mansoni region Uganda - Stothard et al., (2006) Acta Tropica 97:219

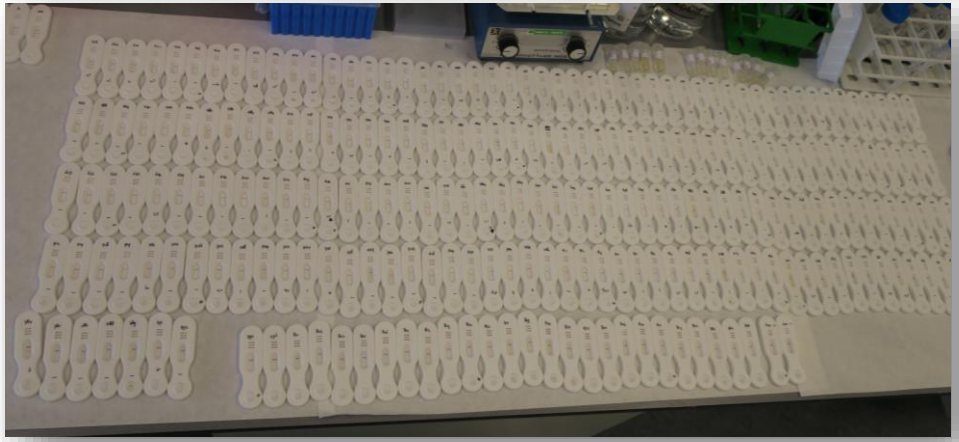
Kittur *et al.,* (2016)





See: http://rapid-diagnostics.com/; Van Dam et al., (2004) J Clin Microbiol; review: Corstjens et al., (2014)

Urine POC-CCA strip assay
Recommended by WHO (2020) for *S. mansoni*



Surveys: replacement of stool microscopy by POC-CCA

Urine test; sensitivity > 1x stool microscopy

S. mansoni ; variable sensitivity for other species

Endemic settings: surveys, mapping, monitoring after treatment

Spec ≈98% (batch variation, QC needs)

- Specificity challenges with <9M infants and pregnant women
- Standardised reading procedures essential (reference G-scores)

o.a. Stothard *et al.,* (2006); Kildemoes *et al, (*2017); M. Casacuberta-Partal et al.,(2019, 2021)

Urine POC-CCA strip assay
Test & treat studies, individual case detection

Govert van Dam and team

Implementation research in Madagascar and Gabon

www.freebily.eu

freeBILy
Schistosomiasis RDT implementation

EDCTP

Non-endemic diagnostics????

Several studies, conflicting findings

=> Migrants with chronic *S. mansoni*

LUMC:

- CoHSI-model, not sensitive enough

o.a. Chernet *et al.,* (2017); Marchese *et al.*, (2018) Beltrame *et al.,*(2017); Hoekstra *et al.,* (2020, submitted)

Ultra-sensitive UCP Lateral Flow CAA assay

Up-Converting reporter Particle technology

Sample pre-treatment

Mix serum or urine with equal volume 4% (w/v) TCA

Spin

(Concentrate supernatant)

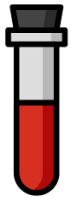
Spin

Add 20 µL supernatant to 100 µL assay-buffer (UCP-McAb)

Mix

Incubate 1 h at 37 °C, shaking

4 mL

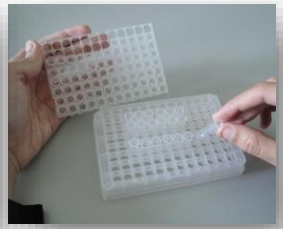


0.5 mL

Govert van Dam and team

Paul Corstjens and team

Optional: dry reagents



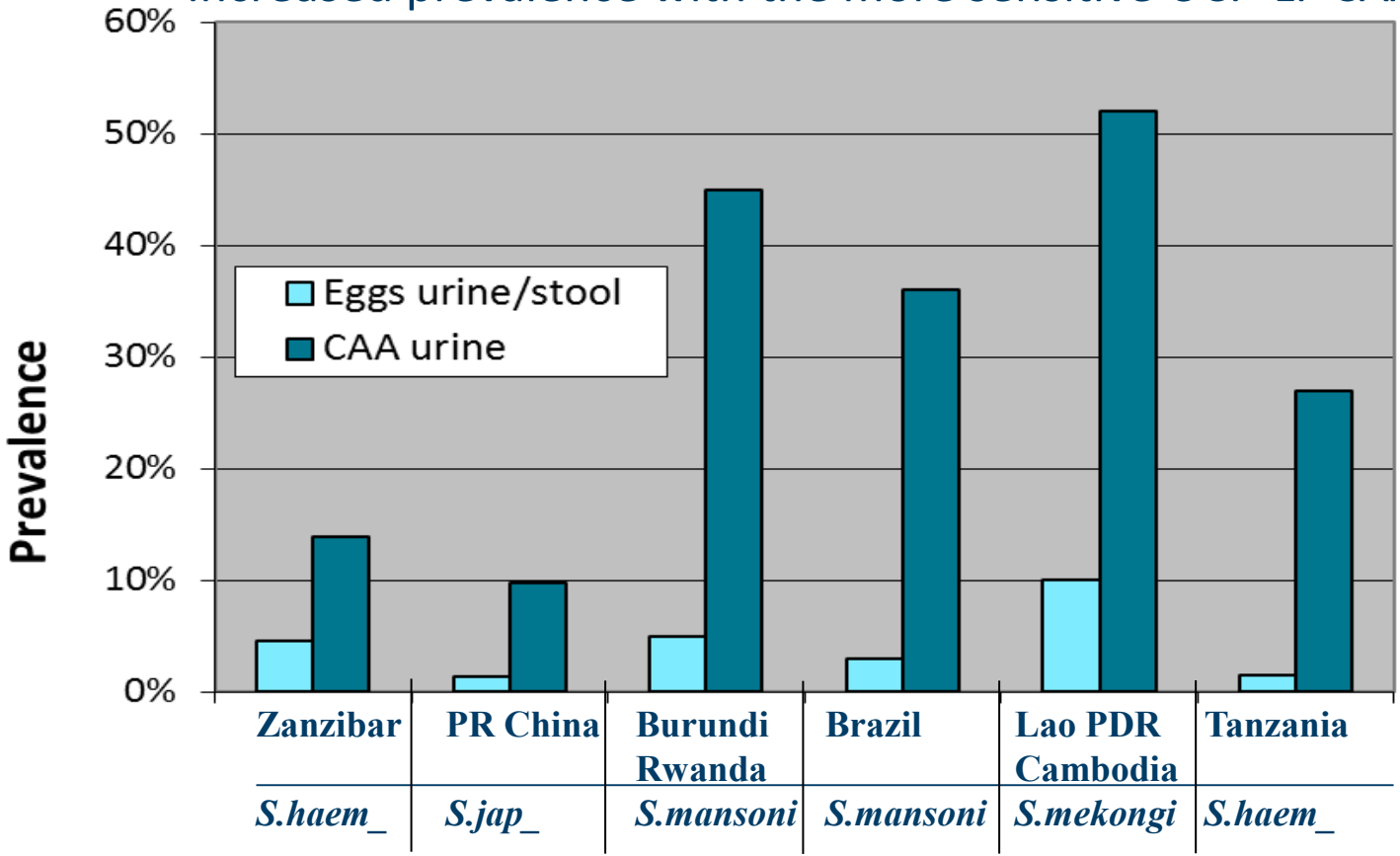
Incubate

Add LF strips, run for 1 h

Read strips, quantitate AWA-TCA dilutions as reference standard

Real Prevalence of Infection ??

Low endemic settings by egg count:
Increased prevalence with the more sensitive UCP-LF CAA



o.a., Knopp et al., PLosNTD (2015); Van Dam et al., Corstjens et al. (2017, 2018, 2019,2020)

Repeated doses of Praziquantel in Schistosomiasis Treatment (RePST) in children from Côte d'Ivoire

Govert van Dam and team

www.repst.org




Cure Rate (CR)

	Kato-Katz	POC-CCA	CAA (urine)	PCR (stool)
Standard (n=70)	**68%** (95% CI 57.1-77.6)	**31%** (95% CI 23.4-40.2)	**23%** (95% CI 16.6-31.1)	**48%** (95% CI 36.1-59.6)
Intense (N=83)	**86%** (95% CI 75.4-92.4)	**36%** (95% CI 26.4-46.1)	**20%** (95% CI 14.3-28.3)	**78%** (95% CI 67.6-86.0)

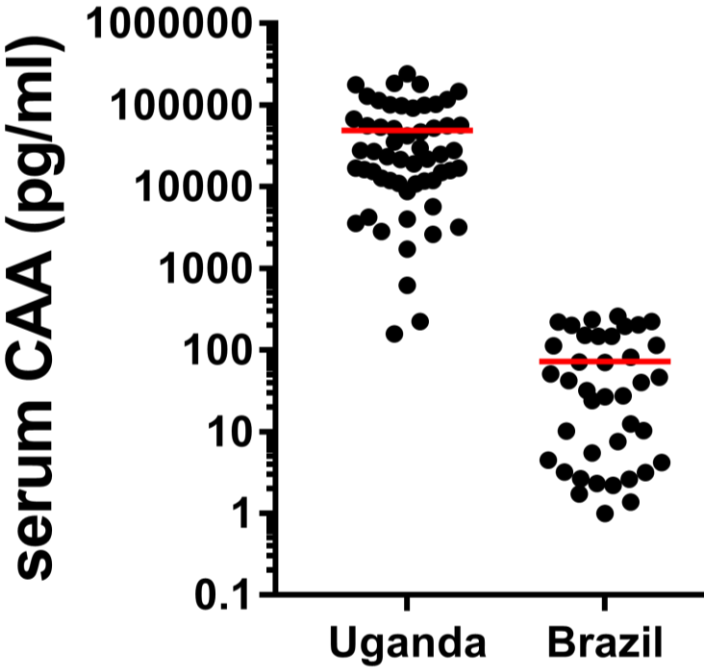
Intensity Reduction Rate (IRR)

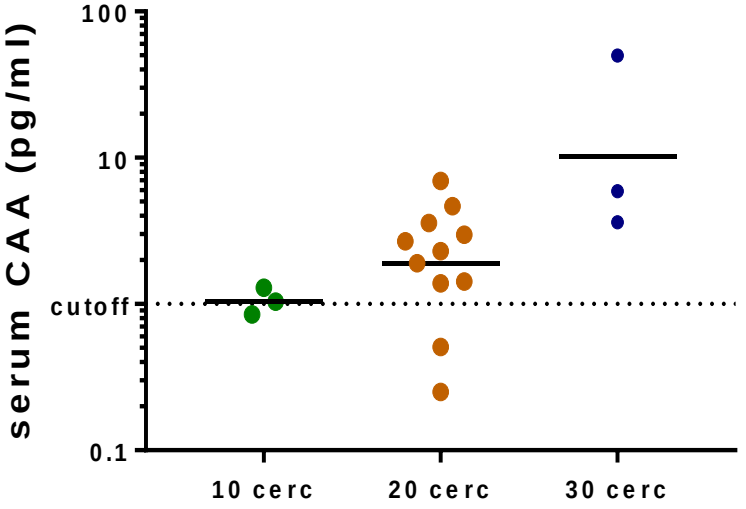
	Kato-Katz			POC-CCA			CAA (urine)			PCR (stool)		
	Pre	Post	IRR	Pre	Post	IRR	Pre	Post	IRR	Pre	Post	IRR
Standard (n=70)	298 (epg)	46 (epg)	**83%**	9172 (pg/ml)	4265 (pg/ml)	**54%**	352 (pg/ml)	59 (pg/ml)	**89%**	61081 (Arbitrary units)	10249 (Arbitrary units)	**83%**
Intense (N=83)	243 (epg)	3 (epg)	**95%**	8488 (pg/ml)	3180 (pg/ml)	**63%**	338 (pg/ml)	26 (pg/ml)	**93%**	37085 (Arbitrary units)	317 (Arbitrary units)	**99%**

Hoekstra *et al.*, (2020) PLoS NTD & Hoekstra *et al.*, manuscript in preparation; ISNTD presentation: https://youtu.be/f1OoEJALth4

CAA levels in serum – different populations

Endemic regions vs Controlled Human Infection Model

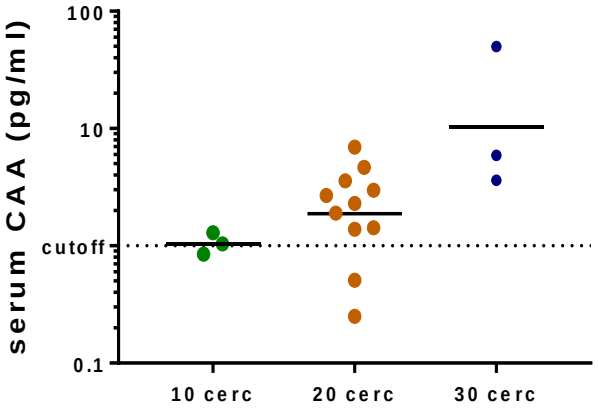


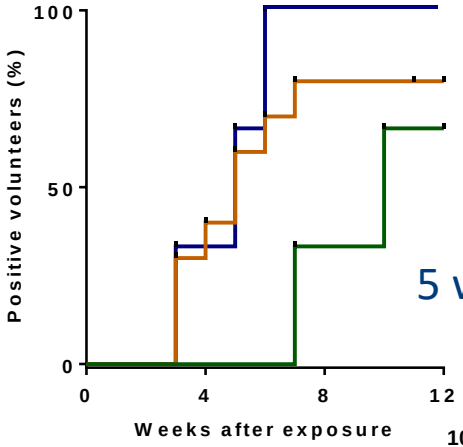


Van Dam et al., Corstjens et al. (2017, 2018, 2019,2020) Langenberg, Roestenberg *et al.,* (2020) Nature Medicine 26(3): 326-32

CoHSI – model
CAA in serum to monitor active infection

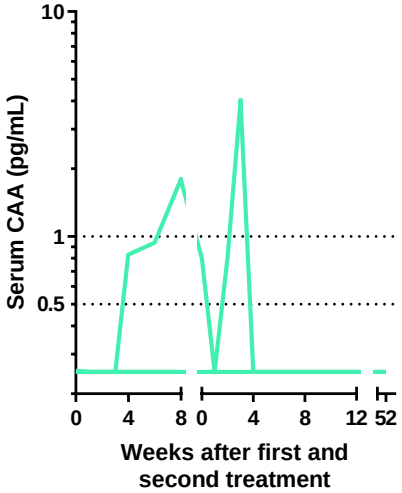
80% infection rate

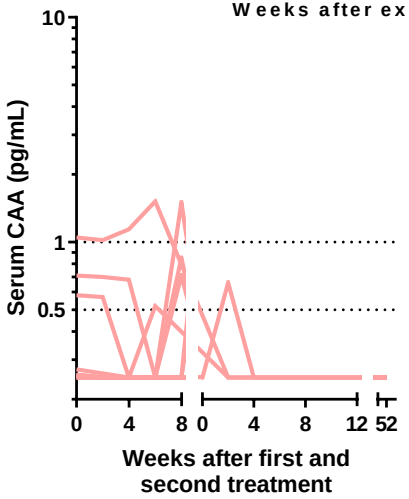


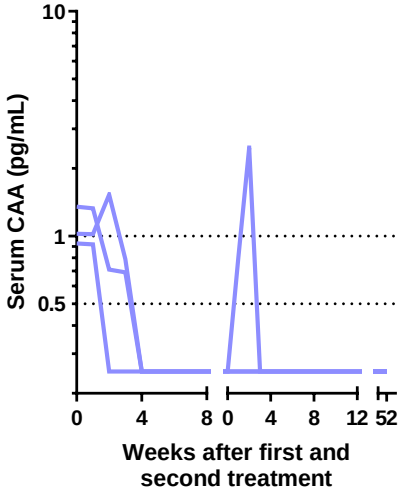


5 weeks prepatent period

1. all: PZQ 40 mg/kgbw (2x20)
2. In 6 CAA levels persisted 3-6 weeks AT (43%): 60 mg/kgbw

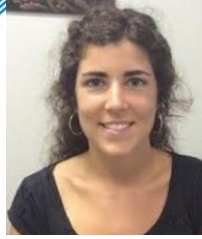






Langenberg, Roestenberg *et al.*, (2020) Nature Medicine 26(3): 326-32

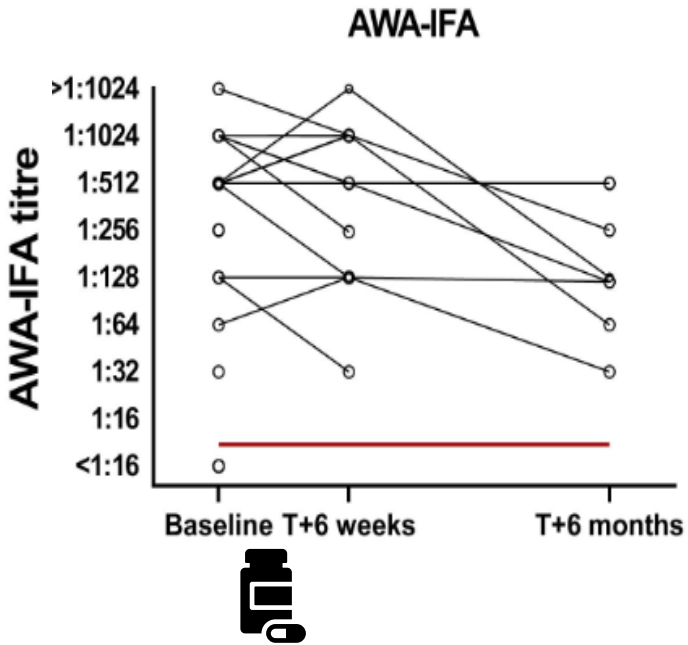
Circulating Anodic Antigen detection reflecting active infection

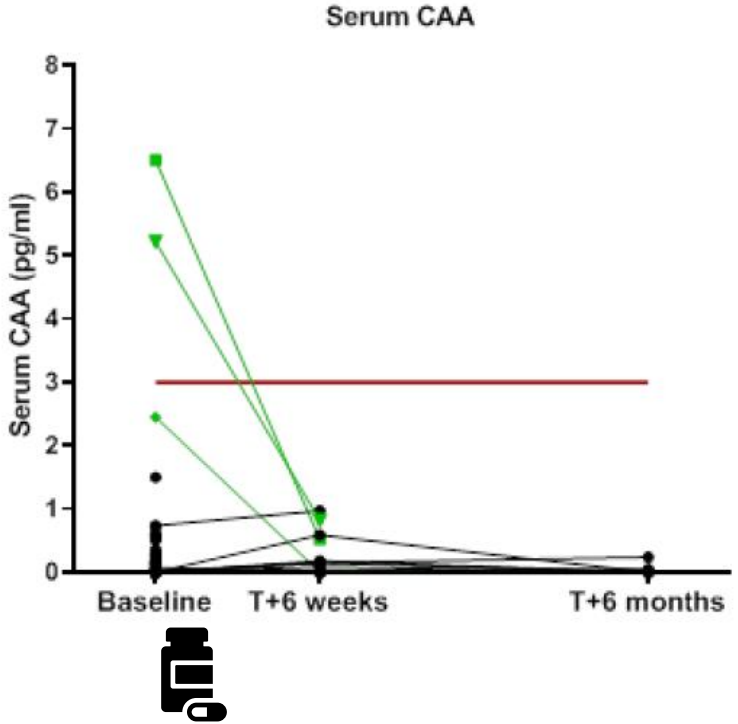


PhD Miriam Casacuberta-Partal

Antigen-based diagnosis of *Schistosoma* infection: a prospective study in 106 Dutch travellers

22 Ab positive – 2 serum CAA (dry assay) positive



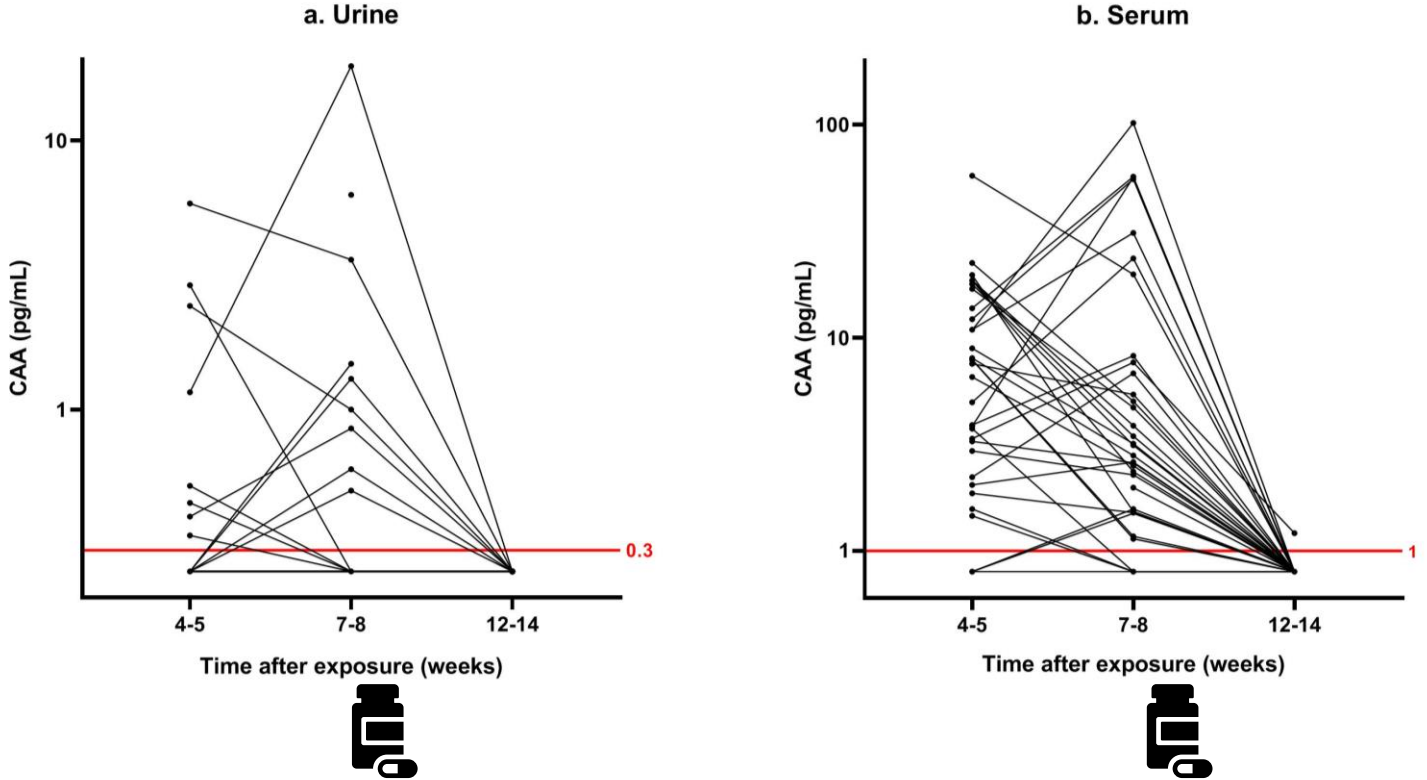


Circulating Anodic Antigen detection reflecting active infection

INSTITUTE OF TROPICAL MEDICINE ANTWERP

PhD Pytsje Hoekstra

Early diagnosis and follow-up of acute schistosomiasis
in a cluster (n=34) of infected (*S. haematobium* complex)
Belgian travellers (exposed in South Africa)



Cnopps *et al.*, (2020) Clin Infect Dis ; Hoekstra *et al.*, (2021) Travel Medicine and Infectious Disease 41 : 102053

Summary: from discovery to implementation
No “one size fits all” test

DNA detecting tests (NAAT)

- Increasingly replaces microscopy; FGS/MGS diagnosis

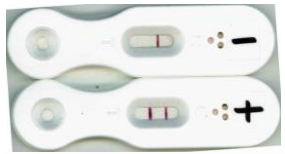
Future: field-friendly test formats? LAMP, RPA etc.

Serology (Ab testing)

- With the right test: early detection in acute schistosomiasis

Circulating antigens

- POC-CCA: S.m. chronic infections (QC in place)
- Ultra-sensitive UCP-LF CAA: early detection, post-PZQ
- UCP-LF CAA for trials/research; pooled samples
- Limited availability
- https://youtu.be/juqIvxN6u0Y



FIND
Because diagnosis matters

frontiers in Tropical Diseases

MINI REVIEW
published: 05 August 2021
doi: 10.3389/fitd.2021.722438

Context-Specific Procedures for the Diagnosis of Human Schistosomiasis – A Mini Review

Pytsje T. Hoekstra, Govert J. van Dam and Lisette van Lieshout*

Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands

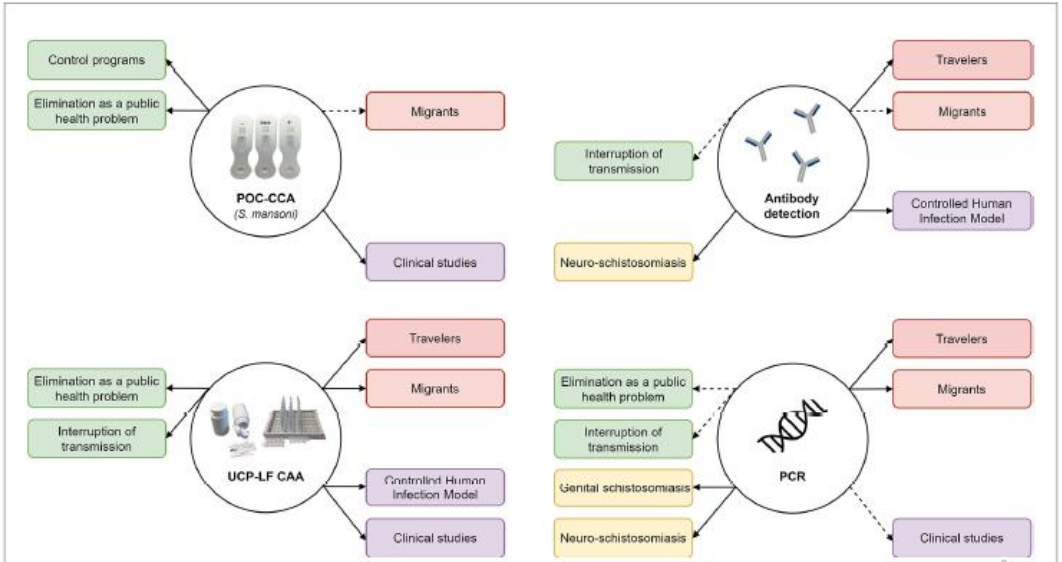
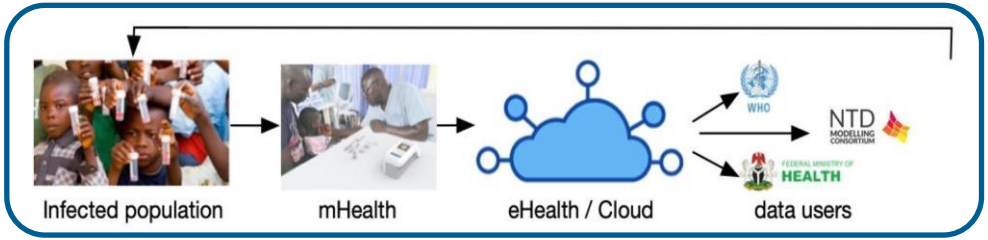


FIGURE 1 | Summary of the applicability of the POC-CCA, UCP-LF CAA, antibody detection and PCR in context-specific settings (solid lines represent first choice diagnostic, dashed lines represent optional or alternative diagnostics). PCR, Polymerase Chain Reaction; POC-CCA, Point-Of-Care Circulation Cathodic Antigen; UCP-LF CAA, Up-Converting Particle Lateral Flow Circulating Anodic Antigen.

Future steps in CCA/CAA diagnostic research

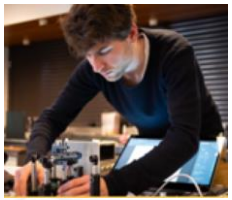
- Basic biology (structure, function)
- Kinetics (host, infection, post-treatment)
- Operational (pooling; data connectivity)



RE – ASSURED – diagnostic requirements

Real-time connectivity
Ease of specimen collection
Affordable
Sensitive
Specific
User-friendly
Rapid and Robust
Equipment-free
Deliverable

Schistoscope – connecting digital microscopy



Noble *et al.* (2020). Deki reader (HIV RDT)

Moving towards novel strip readers

INSPiRED (2021) RDT reader

Connecting point of care data from the field

Leiden University Medical Center

Thank you!

CoHSI - volunteers

Part of the LUMC Schisto / CoHSI team

Prof. Dr. Flu foundation

Netherlands Organisation for Scientific Research

freeBILy
Schistosomiasis RDT implementation

Swiss TPH

TU Delft
Delft University of Technology

Projet RePST

SCI
Schistosomiasis Control Initiative

ESCMID

skml

Natural History Museum

MOLOGIC
BIG IN TINY SCIENCE

INSPiRED

London School of Hygiene & Tropical Medicine

Hospital for Tropical Diseases London

Erasmus MC
Universitair Medisch Centrum Rotterdam

Bill & Melinda Gates foundation

Institute of Tropical Medicine Antwerp

etz

FIND
Because diagnosis matters

Ghent University

MERCK SERONO

star worms
Stop Anthelminthic Resistant Worms

LSTM
Liverpool School of Tropical Medicine
Since 1898

The Task Force for Global Health

Neglected Tropical Diseases Support Center

Partnership for Treating and Preventing Intestinal Worms
ChildrenWithoutWorms