DIAGNOSIS OF SCHISTOSOMIASIS

BY CCA OR CAA DETECTING LATERAL FLOW TESTS

GSA ONLINE SYMPOSIUM
JUNE 2022

GLOBAL SCHISTOSOMIASIS ALLIANCE

Summary

On June 14th 2022 an online symposium was organised by the GSA in collaboration with the LUMC Parasitology team, supported by the INSPiRED project.

The objective of the meeting was to identify shared achievements and challenges in the implementation of the lateral flow tests for the detection of CCA and/or CAA for diagnosing schistosomiasis in endemic regions. The symposium was chaired by Dr Lisette van Lieshout, Leiden University Medical Center (LUMC, Netherlands).

Following an overview of the two antigen-based tests, circulating cathodic antigen (CCA) and circulating anodic antigen (CAA), and their differences presented by Professor Cornelius (Ron) Hokke, LUMC, participants heard experiences, successes, challenges and learnings from three countries;

1. Professor Fernando Schemelzer M. Bezerra, Federal University of Ceará (Siarra), Brazil, shared experiences and challenges of using POC-CCA urine strip tests in Brazil.
2. Dr. Maurice Odiere, Research Scientist, Kenya Medical Research Institute/Safe Water and AIDS project (SWAP), presented data from a comparison study between the POC-CCA urine strip test and the standard Kato-Katz stool test in a schistosomiasis control program in western Kenya.
3. Dr. Jean Coulibaly, Research Scientist, Université Félix Houphouët-Boigny, Côte d'Ivoire, highlighted experiences and challenges of using POC-CCA and UCP-LF CAA tests, presenting data from Cote d'Ivoire, including antigen levels measured in urine samples collected on 3 consecutive days before praziquantel (PZQ) treatment and 7 days following PZQ treatment.

Participants were given the choice of 5 themed break-out rooms to discuss challenges and knowledge gaps, and what is needed to address these gaps. These points were captured in Easy Retro boards and shared with all participants in the main symposium room.

Finally, Dr Sarah Nogaro, Principal Scientist in the NTD team, leading the work on schistosomiasis at FIND, gave the final presentation on the need for a field applicable RDT and the potential challenges, using the example of Schistosomiasis CAA Rapid Diagnostic Test that is currently under development.

This report summarises the facts and challenges presented and discussed in the symposium. The following priority needs and recommendations were identified:

- A commercially available field applicable CAA test
- Guidelines for the verification of different batches of the POC-CCA test
- Establishment of an external quality assessment scheme for CCA and CAA tests
- An ultra-sensitive CAA test available for research purposes
- Follow-up meetings with more exchange of findings between key players and end-users, including a small-scale meeting specifically on POC-CCA discussion points (including manufacturer)

Meeting Report

On June 14th 2022 the GSA in collaboration with the LUMC Parasitology team, supported by the INSPiRED project, organised and hosted an online symposium on the diagnosis of schistosomiasis via the detection of parasite-specific circulating cathodic antigen (CCA) and circulating anodic antigen (CAA). This meeting expanded on an earlier technical meeting by LUMC on CCA and CAA and aimed to share achievements and challenges in the validation and implementation of lateral flow tests, for the detection of CCA and or CAA, for diagnosing schistosomiasis within endemic regions.

The objectives of the online symposium were to:

- Identify shared achievements and challenges in the validation and implementation of lateral flow tests for the detection of CCA and/or CAA for diagnosing schistosomiasis within endemic regions
- To learn about other's experiences with the detection of CAA/CCA; and to discuss current challenges and needs
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In total 146 people registered, including those on the waiting list, and 81 participants joined the online symposium on the day, approximately 40% from Schistosoma endemic countries. Simultaneous English and French interpretation was available during the main sessions and one of the five breakout rooms was in French.

Background

Microscopy, i.e. the detection of Schistosoma eggs in stool or urine, is the classical method for diagnosing schistosomiasis. This method is highly specific, but it requires skilled personnel, is highly observer and operator dependent and is known for its low sensitivity.

The fact that low intensity infections are often missed leads to an overestimation of cure rates following interventions such as preventative chemotherapy using praziquantel (PZQ) through mass drug administration (MDA).

Diagnosing schistosomiasis via the detection of parasite-specific circulating antigens has been shown to be a valuable alternative. Well studied are the circulating cathodic antigen (CCA) and circulating anodic antigen (CAA), two adult worm gut-associated carbohydrate antigens which are continuously regurgitated by viable parasites into the blood circulation and thereafter excreted via urine.

For the detection of CCA in urine there is a commercially available point-of-care test, which is currently recommended by the WHO for the diagnosis of intestinal schistosomiasis as an alternative to stool microscopy. Numerous studies have shown the POC-CCA urine strip test to be at least as sensitive as microscopy based on a single stool sample, but at the same time there are concerns about the specificity and reproducibility of the test.

Detection of CAA via a lateral flow (LF) test using Up-Converting Particle (UCP) technology has shown to be a highly specific way of diagnosing all Schistosoma species. This test, similar to the POC-CCA urine strip test, has been developed by the Leiden University Medical Center (LUMC, the Netherlands). In combination with the appropriate concentration device, the UCP-LF CAA assay can detect extremely low antigen concentrations in either serum or urine. This laboratory-based test has been used by several research groups, including within endemic regions, but is not commercially available.

Summary points of the main presentations

For slides see Appendix 1 (pdf).

Professor Cornelius (Ron) Hokke, LUMC, Leiden Netherlands introduced the two antigens, explained the differences between CCA, and CAA and described the major characteristics of the POC-CCA urine strip test and the UCP-LF CAA test.

Professor Fernando Schemelzer M. Bezerra, Federal University of Ceará (Siarra), Brazil
Described differences seen in the diagnostic performance of tests produced in South Africa and tests produced in Brazil. Use of G-scores alone seems insufficient without appropriate standards. Following the presentation, questions were asked about further standardisation of the test. It was concluded that this is partly the responsibility of the company. Users can ask the test manufacturer for a quality control report. However, it was highlighted that users need to implement the appropriate quality control steps. Validation assays for different batches are relevant. The need for a standardised reference test and of an external quality assessment scheme (EQAS) was discussed. Cost is a relevant issue, as the POC-CCA test in Brazil is $5 in comparison to $1-2 for the Kato-Katz.

Dr. Maurice Odiere, Research Scientist, Kenya Medical Research Institute/Safe Water and AIDS project (SWAP)
Presented data from a comparison study between the POC-CCA urine strip test and the standard Kato-Katz stool test in a schistosomiasis control program in western Kenya.

An overall correlation was seen between intensity of infection based on microscopy and test-line intensity of the POC-CCA test, but at the same time the number of mismatches was surprisingly high. More than 50% of microscopy negatives were found to be positive in the POC-CCA urine test although most had trace or 1+ band intensity only. Consequently, in the follow-up surveys little effect was seen when looking at the percentage of POC-CCA positives, while the number of microscopy positives clearly declined. The batch-to-batch variation in the POC-CCA urine strip test was identified as the most likely explanation. In the discussion similar experiences were mentioned by other groups. The importance of appropriate standardisation was again raised, but in addition possible alternative explanations were brought forward, including biological reasons why CCA would remain detectable following MDA rounds, while egg counts decrease. Data modelling of egg counts and POC-CCA outcomes resulted in more insight. Studies are currently in progress where additional diagnostic tests, including PCR, are being used to get a better picture of the diagnostic value of each test.

Dr. Jean Coulibaly, Research Scientist, Université Félix Houphouët-Boigny, Côte d'Ivoire

Presented data on antigen levels measured in urine samples collected on 3 consecutive days before PZQ treatment and 7 days following PZQ treatment. Some variation in antigen level was observed. Questions included whether this variation was similar when looking at the egg excretion. Discussions highlighted that an antigen negative test with a positive stool microscopy is generally more difficult to interpret than the other way around. Another point of discussion centred on the clearance of the antigen following treatment and that the starting point at baseline probably also influences the speed of becoming antigen negative.

Summary break-out rooms and final presentation from FIND

For Easy Retro Board per break-out session, see Appendix 2.

Participants were invited to join one of 5 break-out rooms to discuss in detail specific topics of CCA and CAA diagnostics:

1. Quality Control of CCA/CAA
2. Post treatment CCA/CAA monitoring
3. Quantitative CCA/CAA data, needs and challenges
4. CCA and CAA as a research tool (in endemic settings), needs and challenges
5. Contrôle de qualité et données quantitatives

Each break-out room discussed the following key questions:

- What are the challenges and gaps?
- What should be the targeted outcome/goal?
- What is needed to address these gaps/achieve these outcomes?

BO1 Quality Control of CCA/CAA

Dr Evan Secor presented on POC CCA and CAA test formats, need of transparency from test manufacturers and questions regarding whose responsibility it is to do quality control (QC) – the manufacturer, end-user or independent stakeholders such as WHO? Dr Andrew Edielu discussed research on PZQ in pre-schoolers and experiences with variations in POC-CCA performance.

The group discussed the role of the manufacturer of the POC-CCA test and that more transparency in the manufacturer's QC process would help. A suggestion was made to start an open data platform to share data and experiences per lot number of POC-CCA tests, and always publish lot numbers.

BO2 Post treatment (MDA) monitoring and evaluation by CCA/CAA, what are the challenges?

Dr Steffi Knopp presented on diagnostics needs for M&E as programmes move to elimination as a public health problem and interruption of transmission, when the majority of infections will be of low to very low intensity. The group discussed the difference between using prevalence and intensity of infection as an indicator for transmission and whether pooling of samples could be an efficient approach for precision mapping. When moving to a stop MDA scenario, the need to monitor post-treatment to avoid resurgence and to be able to react to outbreaks, raises the question of what diagnostics to use (point-of-care, high throughput, focus on sensitivity or rather specificity).

BO3 Quantitative CCA/CAA data, needs and challenges

Dr Adriko Moses and Dr Elias Kabbas Pinango presented on their experience of using the CCA tests and G-scores for quantification within their work in endemic areas of Uganda. There was a lot of discussion about the pros of the G-score system but also the challenges faced in the field. This included the need to protect the G-scores in a waterproof system to prevent them getting wet and affected by humidity; to have a system that makes it easier to compare the bands e.g. having the bands being printed on one piece of paper that can easily be compared to the test being done. Having more dilutions would also be beneficial as it was found that many clinical results were often between two G-scores.

Saturation of the CCA tests was also discussed, to know at what worm burden the tests reach their maximum capacity. A better understanding of how the G-scores actually relate to intensity of infection is needed. The "Use Case" for the CCA test was discussed and suggestions were made for two types of antigen tests, one to determine the prevalence, the other for intensity of infection.

BO4 CCA and CAA as a research tool (in endemic setting), needs and challenges

Emmanuella Driciru presented findings from Uganda on treatment outcomes from the PIP POPVAC trials using the UCP-LF CAA assay. Under supervision of the LUMC team it was technically possible to implement the test in Uganda, but there were also several challenges, such as the effect of humidity on strip quality, time consuming strip reading procedures and complexity of extracting data from the strip reader. An easier to use (commercially available) CAA test would help. John Archer presented data of a study performed in Malawi. With proper calibration and reference samples the POC-CCA could be interpreted in a quantitative way. False positives were noticed with the POC-CCA test, while PCR missed some microscopy positive infections. The group discussed the need for a generally accepted reference standard. In populations with low infection intensities some level of diagnostic discrepancy will always occur, but this should be minimised by (i) improved test standardisation, (ii) less batch-to-batch variation by the manufacturer and (iii) the implementation of an EQAS.

BO5 Contrôle de qualité et données quantitatives

Dr Josianne Honkpehedji presented on data the use of the UCP-LF CAA assay in Gabon (S. haematobium). Although the test has many advantages, it requires a well-equipped lab and well-trained staff.

Dr Ruffin Assare shared data on the POC-CCA test and the UCP-LF CAA test in Côte d'Ivoire. A more field-friendly CAA test format is needed. A mobile phone-based reader for the POC-CCA test was suggested, also to assist in the interpretation of the “traces”. More exchange of data and experiences between laboratories in endemic countries using the antigen tests was discussed. The group discussions also centred on QC aspects of the tests and how to interpret test discrepancies.

Dr Sarah Nogaro, Principal Scientist in the NTD team, leading the work on schistosomiasis at FIND, gave the final presentation on the need for a field applicable RDT and the potential challenges, using the example of the Schistosomiasis CAA Rapid Diagnostic Test (currently under development), which uses a finger prick blood sample. Three challenges were described:

1. In the absence of a gold standard, it is important to determine a suitable (composite) reference test when evaluating the performance of new tests. What should those be? For the field evaluation of the CAA RDT prototype, the performance was measured against three days microscopy, PCR and the UCP-LF CAA assay. Each of these currently available diagnostic tests have their advantages but also its own limitations.
2. How to determine intensity of infection based on CAA levels and how do these relate to eggs, especially if this will drive treatment frequency? Work done by LUMC shows that there are differences in CAA secretion for the different schistosomes. Determining the most appropriate cut-off value is thus important.
3. What is the right access and regulatory pathway for these new tests? New initiatives are being setup like the WHO Coordinated Scientific Advisory committee to provide guidance on the most appropriate way to generate robust evidence with the view of obtaining WHO policy recommendation.

Summary of findings and recommendations

The importance and interest in the topic are shown by the excellent participation. It is vitally important to improve and strengthen diagnostic tests to move to schistosomiasis elimination as a public health problem and eventually achieve interruption of transmission. The new WHO guidelines and recommendations are highly reliant on having good diagnostics in place. The push towards community wide treatment requires that we make the best use of available PZQ, it is essential that we are able to identify areas of high transmission and accurate, affordable diagnostics will be key, as they will also be in areas of low transmission, where it will make sense to move to a test and treat scenario. With such a long history in the laboratory and in the field CAA and CCA have an important role to play in helping to achieve the elimination goals.

The following priority needs and recommendations were identified through the presentations and break-out group discussions:

- A commercially available field applicable CAA test.
- Guidelines for the verification of different batches of the POC-CCA test.
- Establishment of an external quality assessment scheme for CCA and CAA tests.
- An ultra-sensitive CAA test available for research purposes.
- Follow-up meetings with more exchange of findings between key players and end users, including a small-scale meeting specifically on POC-CCA discussion points (including manufacturers).

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Appendix

Appendix 1 (pdf) Slides of the main presentations
Appendix 2 (pdf) Easy Retro Board per break-out session
Appendix 3 (pdf) Symposium impression screen shots

Aim & objectives:

- Identify shared achievements and challenges in the validation and implementation of lateral flow tests for the detection of CCA and/or CAA for diagnosing schistosomiasis within endemic regions (to consider: include use in travel medicine, i.e. imported infections)
- To learn about other's experiences with the detection of CAA/CCA; to discuss (hear opinions and experiences) current challenges and needs.

Date 14 June 2022
Time 14:00 – 17:15 (CEST)
Platform: Online event using Zoom Events and live interpretation in English and French.

Attendees:

- In total 146 people registered, including the waiting list
- In total 81 people attended the Zoom Events, approximately 40% of them based in Schistosoma endemic countries.

Programme:

- Overview of topic - Ron Hokke

- Presentations from country representatives
  - Fernando Bezerra (Brazil)
  - Maurice Odiere (Kenya)
  - Jean Coulibaly (Côte d'Ivoire)

- Parallel sessions in breakout rooms:
  - BO1- QC aspects of CCA/CAA detection; from batches to readings. Need for certification? Need for EQAS? (English) Mediator: Sarah Nogaro
  - BO2- Post treatment (MDA) monitoring and evaluation by CCA/CAA, what are the challenges? (English) Mediator: Maurice Odiere
  - BO3- Quantitative CCA/CAA data, needs and challenges (English) Mediator: Jessica Clark
  - BO4 - CCA and CAA as a research tool (in endemic setting), needs and challenges (English) Mediator: Ron Hokke
  - BO5- Contrôle de qualité et données quantitatives (Francais) Mediator: Louis-Albert Tchuem Tchuente

- Report back from parallel sessions

- Rapid Diagnostic Tests – requirements and challenges - Sarah Nogaro

- Wrap up and close

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Design and editing by Kat Gulyas Global Health Communications Consultancy