# Detection of schistosome circulating antigens CCA and CAA: diagnostic test interpretation and application

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2

# Chapter 2

## An innovative and user-friendly scoring system for standardized quantitative interpretation of the urine-based point-of-care strip test (POC-CCA) for the diagnosis of intestinal schistosomiasis: a proof-of-concept study

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# ABSTRACT

The point-of-care strip assay for the detection of the schistosome Circulating Cathodic Antigen (POC-CCA) in urine has shown to be a user-friendly and sensitive alternative to stool microscopy for the diagnosis of *Schistosoma mansoni* infections. However, visual scoring of the test is by definition observer dependent and leads to discussion about the qualitative interpretation, in particular in low intensity infections when test lines tend to be weak. In order to standardise visual scoring, an innovative approach for semi-quantitative interpretation of the POC-CCA cassettes, called G-scores, was developed and evaluated. Urines (n=110) from a *S. mansoni* endemic area were used to evaluate this new approach. Test lines of the POC-CCA were visually compared against the G-scores, i.e., a series of artificial cassettes containing inkjet-printed strips of different intensities in order to grade the POC-CCA test line on a scale of 1 to 10. A significant positive correlation (0.660, $p<0.001$) was observed between G-scores and eggs per gram of faeces. This proof-of-concept study demonstrates the usefulness of the G-scores for standardising the visual scoring of the POC-CCA urine strip assay. Several research groups have already indicated an interest in the G-score for their field work. Further distribution of the cassettes, in particular when provided in combination with a reference standard, will assist the wider schistosomiasis community in dealing with issues like batch-to-batch differences and interpretation of trace readings.

# INTRODUCTION

The point-of-care Circulating Cathodic Antigen (POC-CCA) urine test is a rapid diagnostic test (RDT) for the qualitative detection of an active *Schistosoma mansoni* infection. It detects the schistosome-specific, gut-associated excretory antigen CCA, which is regurgitated by (juvenile) worms into the blood circulation of the infected host and excreted in urine [1]. The commercially available test is highly user-friendly. Being urine-based, it also makes sample collection more straight forward than stool-based diagnostic methods, thereby increasing the compliance when collecting samples [2,3].

According to the manufacturer's instructions, any visible test line seen 20 minutes after the application of urine is considered positive. However, many studies have reported a semi-quantitative outcome, namely trace, 1+, 2+, and 3+, showing a positive correlation between increasing test line intensity and faecal egg counts [4, 5, 6]. However, there is a need for standardisation of the visual reading, which is by definition subjective. Especially in areas where most of the cases harbour a low intensity infection, test interpretation is crucial, as it decides whether an individual is still infected or not [7].

In order to standardise semi-quantitative visual scoring of the POC-CCA, a graded, standalone and robust scale was developed. This proof-of-concept study presents in detail the development and evaluation of this colour scale, called G-scores, which consist of 10 POC-CCA cassettes with inkjet-printed strips of different intensities to allow scoring on a 1 to 10 scale (G1-G10), see Figure 1. The scale attempts to mimic the range of colour intensity that can be observed, allowing less reader dependency.

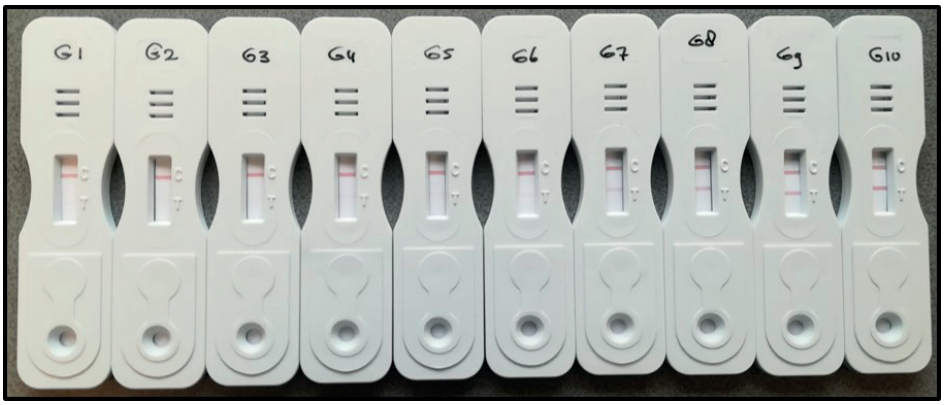


**Figure 1.** The G-scores; a set of 10 POC-CCA cassettes consisting of artificially produced strips with different test line intensities. The colours depicted here might differ from the actual printed cassettes. Therefore, this figure should not be used as a replacement of the G-score.

# MATERIALS AND METHODS

## 2.1 Manufacturing of G-scores

A Canon inkjet-printer model Mx870 was used to print artificial test and control lines with different intensities on 160mg Canon A4 paper. Strips were manually cut and placed into an empty POC-CCA cassette. To check the reproducibility of different printouts as well as the stability in different settings, the artificial cassettes were read using a portable Qiagen ESEQuant Lateral Flow reader, which measures the intensity of the test line based on colorimetric detection resulting in a numerical output. The G-scores are portable and easy-of-use. Their storage conditions are simple by keeping them in dry and dark environments to prevent colour fading due to light.

## 2.2 Evaluation of G-scores in urine samples from an endemic setting

A set of banked urine samples (n=110) with matching Kato-Katz (KK) data, available from a previously published study focusing on the diagnosis of schistosomiasis, was used to demonstrate the use of the G-scores [8]. The POC-CCA test (batch number: 170622073, expiration date: 06/2019) was performed according to the manufacturer's instructions (Rapid Medical Diagnostics, Pretoria, South Africa). After 20 minutes cassettes were read by comparing the intensity of the test line with the G-scores (Figure 2) and then selecting the G-score that best matched the intensity of the test line, resulting in a G-score ranging from G1 to G10. Subsequently, G-scores where recoded into the more widely used visual categories using a conversion table, indicated in Table 1. Four urine reference samples with known antigen concentration, named S-series (S0, S1, S2 and S3), were used as standards. They consist of negative urines spiked with a known antigen concentration, i.e. 0, 80, 800 and 8000 ng/ml of AWA-TCA (trichloroacetic acid-soluble fraction of *S. mansoni* adult worm antigen (AWA), containing approximately 3% CCA) [9].

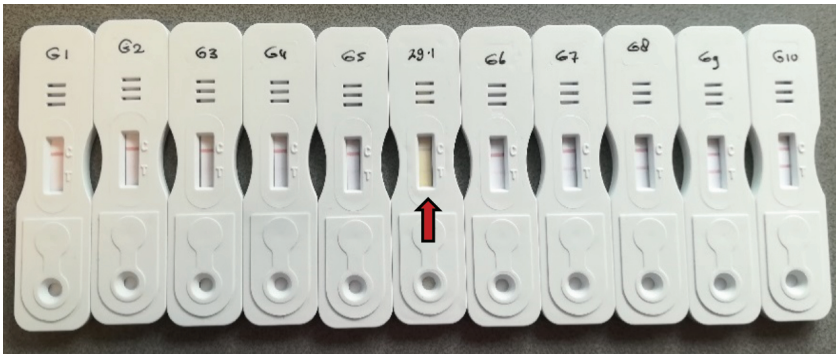


**Figure 2.** Example of scoring a urine sample using the G-scores. The arrow indicates the cassette to be scored, which is placed in between the G-scores that resemble the intensity of the test line in order to select the best matching G-score.

**Table 1.** G-scores and their corresponding visual score.

| G-score | Visual score |
|---|---|
| G1 | 0 |
| G2 | Trace |
| G3 | Trace |
| G4 | 1+ |
| G5 | 1+ |
| G6 | 2+ |
| G7 | 2+ |
| G8 | 3+ |
| G9 | 3+ |
| G10 | 3+ |

## RESULTS AND DISCUSSION

Out of the 110 urine samples, 88 (80%) were POC-CCA positive when including the traces (G2-G3) as positive, and 63 (57%) when including the traces as negative, while stool microscopy found 86 (78%) individuals to be positive. A significant positive correlation was observed between the samples' mean eggs per gram faeces (epg) and their POC-CCA score, either G-score or the recoded visual category (Spearman's rho 0,660 and 0,648; $p<0.001$, respectively) (Figure 3). For the specific POC-CCA batch used here, the S-series gave an outcome of G1, G5, G8 and G10, respectively, which coincide with the expected range of a CCA standard curve when using an ELISA format [10].

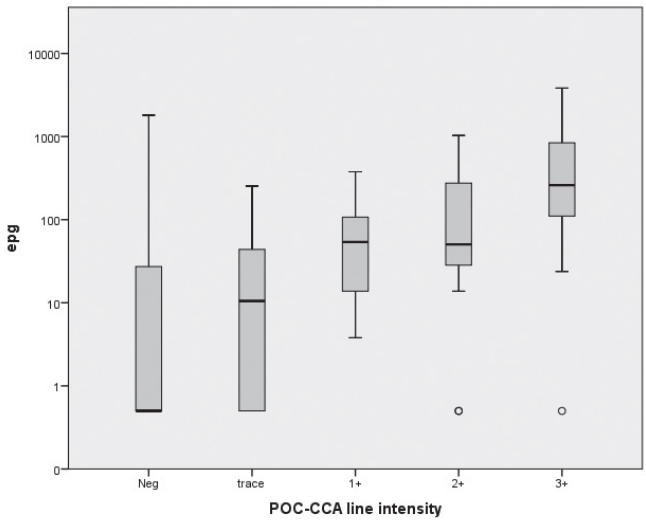


**Figure 3.** Boxplot representing correlation between egg per gram faeces (epg) by Kato-Katz smear and line intensity by POC-CCA. For the purpose of log-transformation a value of 0.5 was added to all data. The 5 POC-CCA commonly used categories correspond to the converted G-scores using Table 1. There is a significant positive correlation between epg and POC-CCA line intensity (Spearman's rho 0.660, $p<0.001$).

## Conclusion

In conclusion, the present proof-of-concept study introduces a visual support tool, called G-scores, for standardisation of scoring the intensity of the POC-CCA test line and demonstrates its applicability. Being user-friendly, the G-scores can be used both in laboratory settings as well as in the field and several schistosomiasis research groups have already shown an interest is using these cassettes [11]. Based on these enthusiastic responses the G-scores, with the accompanying S-series and SOPs, have been made available on request. It is anticipated that wider use of the G-score with the accompanying S-series will help the schistosomiasis community in dealing with issues like batch-to-batch differences and interpretation of trace readings. In the long run, this could be combined with image processing algorithms such a standalone optical reader or a phone application specifically designed to assist the readouts.

## Acknowledgements

We would like to thank Dr. Lyndon Mungur for pointing us towards the use of a colour-based intensity chart and to Dr. Edridah Tukahebwa from Vector Control Division, Ministry of Health, Uganda and Prof. Dr. Birgitte Vennervald, University of Copenhagen, Denmark for allowing us to make use of the archived urine samples for this proof-of-concept study.

# REFERENCES


1. van Dam, GJ., Wichers, JH., Ferreira, TM., Ghati, D., van Amerongen, A., Deelder, AM., 2004. Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. J Clin Microbiol. 42(12):5458-61.
2. Bergquist R., 2013. Good things are worth waiting for. Am J Trop Med Hyg. 88(3):409-10.
3. Utzinger, J., Becker, SL., van Lieshout, L., van Dam, GJ., Knopp, S., 2015. New diagnostic tools in schistosomiasis. Clin Microbiol Infect. 21(6):529-42.
4. Coulibaly, JT., N'Gbesso, YK., Knopp, S., N'Guessan, NA., Silue, KD., van Dam, GJ., 2013. Accuracy of urine circulating cathodic antigen test for the diagnosis of *Schistosoma mansoni* in preschool-aged children before and after treatment. PLoS Negl Trop Dis.7(3):e2109.
5. Dawson, EM., Sousa-Figueiredo, JC., Kabatereine, NB., Doenhoff, MJ., Stothard, JR., 2013. Intestinal schistosomiasis in preschool-aged children of Lake Albert, Uganda: diagnostic accuracy of a rapid test for detection of anti-schistosome antibodies. Trans R Soc Trop Med Hyg. 107(10):639-47.
6. Kittur, N., Castleman, JD., Campbell, CH., King, CH., Colley, DG., 2016. Comparison of *Schistosoma mansoni* Prevalence and Intensity of Infection, as Determined by the Circulating Cathodic Antigen Urine Assay or by the Kato-Katz Fecal Assay: A Systematic Review. Am J Trop Med Hyg. 94(3):605-10.
7. Colley, DG., Andros, TS., Campbell, CH, Jr., 2017. Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs? Infect Dis Poverty. 6(1):63.
8. Tukahebwa, EM., Magnussen, P., Madsen, H., Kabatereine, NB., Nuwaha, F., Wilson, S., 2013. A very high infection intensity of *Schistosoma mansoni* in a Ugandan Lake Victoria Fishing Community is required for association with highly prevalent organ related morbidity. PLoS Negl Trop Dis.7(7):e2268.
9. van Dam, GJ., Bergwerff, AA., Thomas-Oates, JE., Rotmans, JP., Kamerling, JP., Vliegenthart, JF., 1994. The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis x as repeating unit. Eur J Biochem. 225(1):467-82.
10. Polman, K., Diakhate, MM., Engels, D., Nahimana, S., Van Dam, GJ., Falcao Ferreira, ST., 2000. Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. Trop Med Int Health. 5(8):534-7.
11. Haggag, AA., Casacuberta Partal, M., Rabiee, A., Abd Elaziz, KM., Campbell, CH., Colley, DG., 2019. Multiple Praziquantel Treatments of *Schistosoma mansoni* Egg-Negative, CCA-Positive Schoolchildren in a Very Low Endemic Setting in Egypt Do Not Consistently Alter CCA Results. Am J Trop Med Hyg. 100(6):1507-11.

# SUPPLEMENTARY INFORMATION

**Supplementary Table 1.** Example of egg count seen per G-score category. Variation in range and median egg-excretion per G-score is expected when dealing with different study populations.

| G-score | Visual score* | n | min epg | med epg | max epg |
|---|---|---|---|---|---|
| 1 | Neg | 22 | 0 | 0 | 1810 |
| 2 | Trace | 17 | 0 | 7 | 253 |
| 3 | Trace | 8 | 0 | 28 | 93 |
| 4 | 1+ | 2 | 40 | 47 | 53 |
| 5 | 1+ | 7 | 3 | 53 | 377 |
| 6 | 2+ | 9 | 0 | 143 | 513 |
| 7 | 2+ | 11 | 0 | 43 | 1030 |
| 8 | 3+ | 12 | 27 | 165 | 1323 |
| 9 | 3+ | 10 | 0 | 213 | 3837 |
| 10 | 3+ | 12 | 107 | 517 | 2335 |

*Corresponding visual score for the POC-CCA batch used in this project

## Supplementary SOP.

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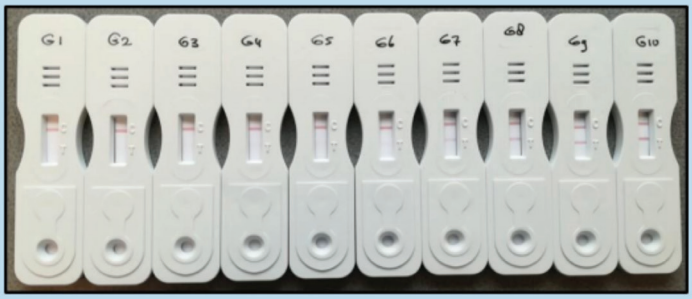
# LUMC QC-kit

*Standard Operating Procedure*

*for scoring POC-CCA™ using the G-scores and S-series*




*Disclaimer: The LUMC QC-kit, including this Standard Operating Procedure (SOP), has been developed by members of the Parasitology department at LUMC. The LUMC QC-kit is made available for research use only. The LUMC team disclaims all liability in reference to any risk when misusing the LUMC QC-kit or SOP.*

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*August 2019*

| Version | Date | Author(s) | Comments |
|---|---|---|---|
| 1.0 | June 2019 | M. Casacuberta Partal, P.T. Hoekstra, D. Kornelis, L. van Lieshout, G.J. van Dam | |
| 2.0 | August 2019 | P.T. Hoekstra | The title and disclaimer have been updated |

# TABLE OF CONTENTS

# 1. SCOPE AND APPLICATION

The point-of-care circulating cathodic antigen (POC-CCA) urine cassette test is a rapid diagnostic test (RDT) for the qualitative detection of an active *Schistosoma* infection, manufactured by Rapid Medical Diagnostics, South Africa [1]. This SOP describes how to perform the POC-CCA test on urine samples in order to determine the intensity of the infection with *Schistosoma* by quantitatively scoring the results using an artificial scoring set (G-scores) as a grading reference to standardize the reading of the results [2].

Please note that POC-CCA tests are not included in the LUMC QC-kit and should be ordered from Rapid Medical Diagnostics separately.

# 2. RESPONSIBILITIES

| Person(s) | Activities |
|---|---|
| Lab technician<br>Preferably two independent technicians | • Perform the POC-CCA urine cassette test for the diagnosis of *Schistosoma* infection according to SOP<br>• Record the results |

# 3. MATERIALS

## 3.1 Materials needed to perform a POC-CCA test

The following materials are needed for a POC-CCA test (Figure 1):

- Urine sample
- POC-CCA urine cassette [1]
- Straw dropper [1] or a micropipette if available

Please note that POC-CCA tests are not supplied with the G-scores and should be ordered from Rapid Medical Diagnostics separately.

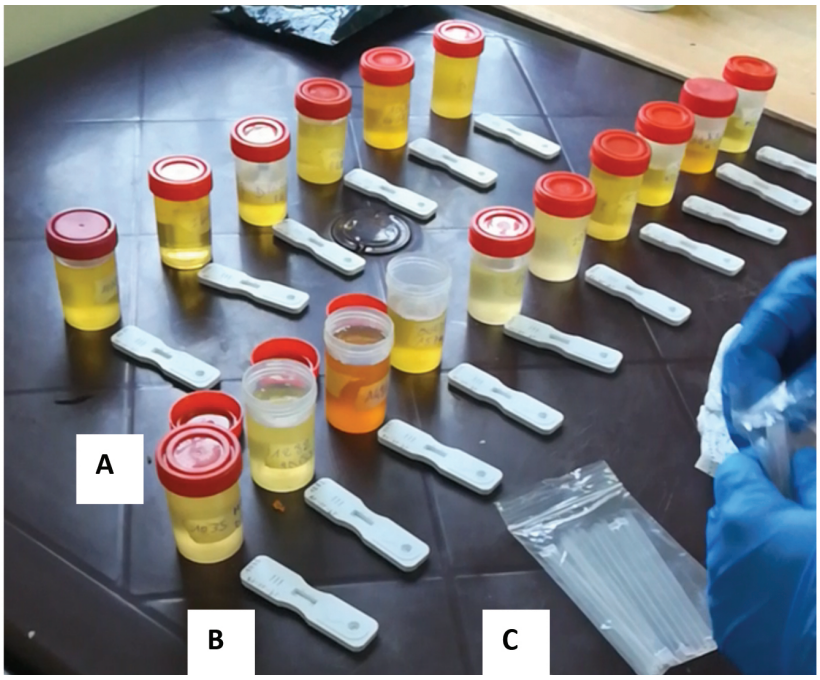


**Figure 2.** (A) Urine samples, (B) POC-CCA cassettes, (C) straw droppers.

## 3.2 Additional tool for scoring the POC-CCA

### *3.2.1 S-series*

The S-series are spiked reference samples with increasing CCA concentrations. The S-series consist of 4 reference standards called S0, S1, S2 and S3 (see Figure 2), containing negative urines spiked with a known antigen concentration, i.e. respectively 0, 80, 800 and 8000 ng/ml of AWA-TCA respectively (a trichloroacetic acid-soluble fraction of *S. mansoni* adult worm antigen, containing approximately 3% CCA) [3]. The S-series are lyophilized before being distributed and as such can be stored at room temperature (maximum 37°C) until use. Reconstitute the lyophilized material by adding 500µl of water (bottle water, tap water or MilliQ). Following reconstitution, store at -20°C or alternatively in a fridge (4-7°C) if no freezer is available, until finished.

It is recommended to test every new batch of POC-CCA cassettes with the S-series and to repeat this at least once every 6 months if a batch of cassettes is used for a longer period.

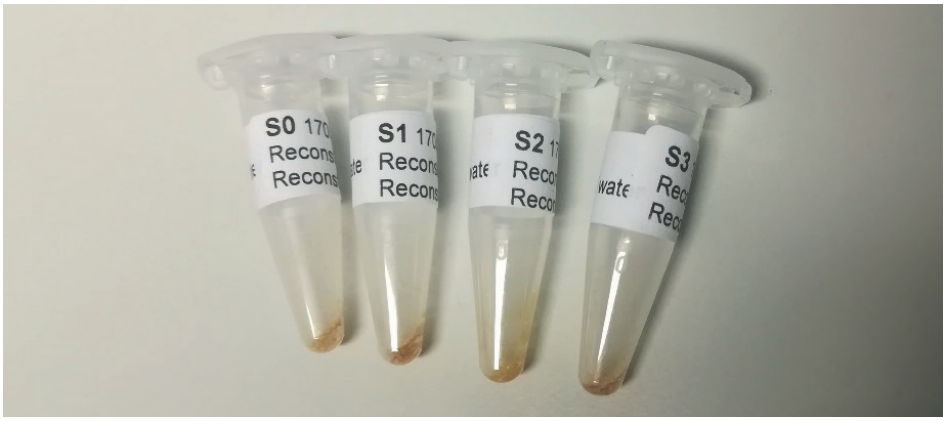


**Figure 3.** S-series in lyophilized format.

### *3.2.2 G-scores*

The G-scores consist of a set of 10 reference cassettes, the so-called G-scores, see Figure 3. The G1 cassette has no test line and therefore it mimics a clear negative result. The G2 and G3 cassette have a fainted test line, called 'trace'. All G-scores from G4 to G10 are considered positive tests. The corresponding visuals scores are given in Table 1.

The G-scores should be stored in a dark and dry place (e.g. drawer). Do not leave the cassettes exposed to light longer than necessary. The G-scores can be used for a period of at least 3 years, assuming they have been stored under the right conditions. Only use the G-score set of cassettes as provided by the LUMC, do not use Figure 3 as an alternative.

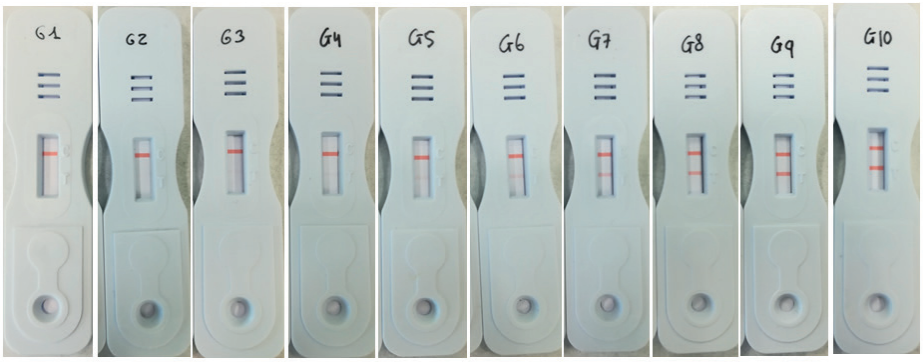


**Figure 3.** Set of G-scores, from left to right: negative test line (G1) to strongly positive test line (G10). The colours depicted here might differ from the actual printed cassettes. Therefore this figure should not be used as a replacement of the G-score cassettes.

**Table 1.** G-scores and their correspondent visual score

| G-scores | Visual scores based on G-scores |
|---|---|
| G1 | 0 |
| G2 | Trace |
| G3 | Trace |
| G4 | 1+ |
| G5 | 1+ |
| G6 | 2+ |
| G7 | 2+ |
| G8 | 3+ |
| G9 | 3+ |
| G10 | 3+ |

# 4. PROCEDURES

## 4.1 Safety

- Handle all samples as potentially infectious (Figure 4).
- Wear gloves and a lab coat during the procedure.
- Practice safety precautions for handling and disposal of infectious materials.

**Figure 4.** Example on handling the urines.

## 4.2 Preparation of the test

1. Ensure the pouches containing the tests are still sealed.
   The silica pouch inside the pouch must be pink/orange (Figure 5), if green/blue then discard the test.
2. Ensure all reagents and the urines are at room temperature (preferably 20-25 °C) before starting the assay.
3. For each new POC-CCA batch, always first read the instructions provided inside the box. If needed, adjust the volume of urine accordingly.
4. Remove the test cassette from the pouch and take a dispensing device from the bag just prior to use (Figure 5). Don't leave the cassette opened on the table for more than 30 min.
5. Label the cassettes according to the ID given from the urine sample (Figure 6A).
6. Record the batch number of used the POC-CCA cassettes. The batch number is printed on the box as well as on each individual pouch, see Figure 6B and C.

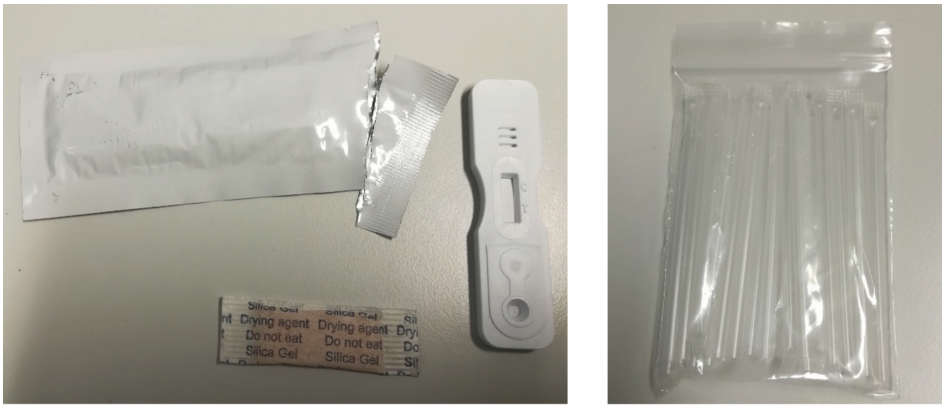


**Figure 5.** (Left) Opened pouch, cassette and normal silica pouch. (Right) dispensing straw dropper.


**Figure 6.** (A) Example of a labelled cassette, (B) Location of the batch number on the POC-CCA box, (C) Location of the batch number (BN) on the pouch.

## 4.3 Assay procedure

1. Homogenize the urine sample by shaking or stirring (Figure 7A).
2. Squeeze the pipette top and insert the tip into the urine sample (Figure 7B).
3. Allow the straw to fill up with urine by gently releasing the straw dropper.
4. Hold the straw dropper at a 45° angle (Figure 7C).
5. Transfer 2 drops of urine into the circular well of the test cassette by gently squeezing the straw dropper. Each drop is equivalent to about 40-50 µL. Alternatively, use a micropipette to transfer 100 µL of urine.
6. Please note that the cassette should be placed on a flat surface**.** (Figure 7C/D).
7. Allow the sample to absorb entirely into the specimen pad (Figure 7C) within the circular well.

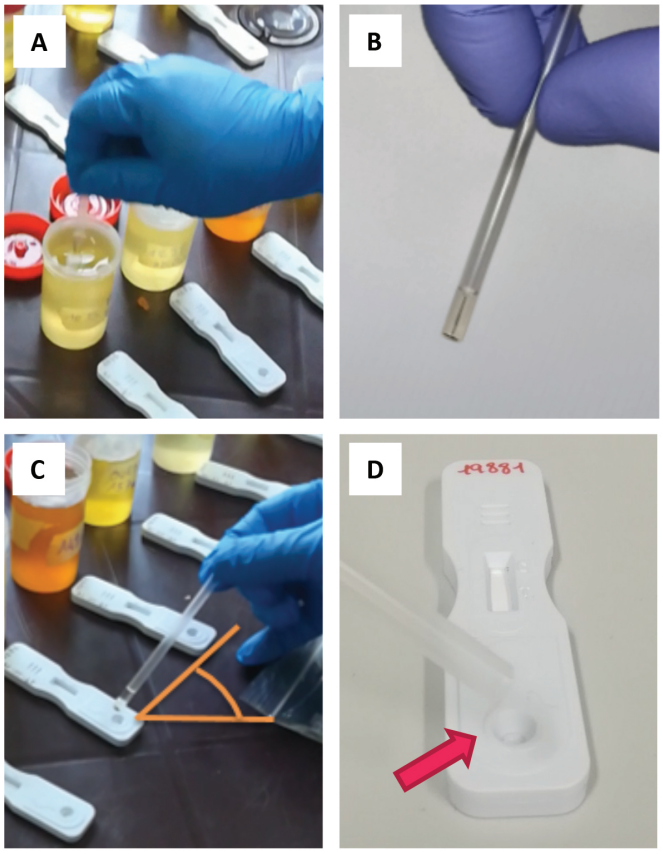


**Figure 7.** (A) Homogenizing a urine sample, (B) squeezing the pipette, (C) Position for the straw dropper in order to transfer the urine to the cassette, (D) specimen pad where the urine sample should be dropped.

8. Once the pipette has been used, discard the pipette with the remaining urine inside and **do not re-use it**.
9. Read the result exactly **20 minutes** after adding the urine onto the cassette.
10. Any results read after ≥**25 minutes** should be considered as **invalid** and must be repeated.
11. The control line must turn pink. If the control line does not have a colour after 20 minutes, the test should be considered as invalid. Also, if the whole strip remains pink, this means that the flow of the strip is not correct and the test should be considered as invalid. Also see Figure 8 for more details.

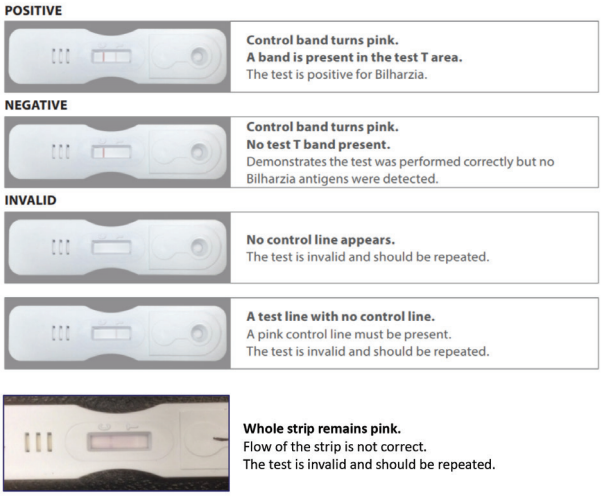


**Figure 8.** Interpretation of results – adapted from the Rapid Medical Diagnostics pamphlet.

## 4.4 Scoring the POC-CCA test result

1. Valid tests will be scored by using the G-scores for an accurate (see Figure 3).
2. Look at the test line (lower line in the cassette) and compare the intensity of the test line to the most similar G-score cassette. Place them next to each other for easier recognition (Figure 9).


**Figure 9.** Scoring a cassette by looking at the test line and comparing with the set of G-scores.

3. Record the G-score of the test line, see 4.6 Documentation of results.
4. It is recommended to take a picture of the cassette at 20 minutes, with or without the corresponding G-score cassette.
5. Translate the G-scores to a visual score of neg, trace 1+ to 3+, see Table 1. This can be done after entering the data into the computer.



## 4.5 Documentation of results

Appendix 1 provides a template of how the results could be recorded. Preferably, the reading of the cassettes, which should always be between 20 and 25 minutes after adding the urine, is done by two independent technicians, each using their own recording sheet.

## 4.6 Quality control

For standardisation of the results, we highly recommend to test each batch of POC-CCA cassettes at least once by using the set of standardized reference urine samples, the S-series (Figure 2). Following reconstitution, the S-series should be tested in the same manner as urine samples, by following the procedure as described in 4.3. The outcome of each S-series test should be noted in the same way as the urine samples (Appendix 1). Overall, the S-series should show a step-wise increase in G-score for each concentration.

In case the S-series does not coincide with the ranges indicated in Table 2, the output of the POC-CCA test should be considered as invalid and we should be contacted.

**Table 2.** Performance of S-series

| S-series | G-score range |
|---|---|
| **S0** | **G1** |
| **S1** | **G3-G6** |
| **S2** | **G6-G9** |
| **S3** | **G10** |

## 4.7 Waste management

Dispose remaining potentially contaminated material and urine according to the local Health and Safety regulations.

## 4.8 Precautions

- All human urine products should be handled as potentially infectious material.
- Testing materials should be disposed of in accordance with local, state and/or federal regulations.
- Care should be exercised to protect the reagents from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of containers, reagents or dispensing equipment can lead to false test results.

### *POC-CCA cassettes:*

- Keep the POC-CCA storage boxes in a dry place.
- Do not reuse POC-CCA cassettes.
- Do not use POC-CCA cassettes if foil pouch is punctured or damaged.
- Never pipette by mouth or allow reagents or patient sample to come into contact with skin.
- Optimal results will be obtained by strict adherence to this protocol. Reagents must be added carefully to maintain precision and accuracy.
- Performing the test outside the prescribed time and temperature ranges will result in an invalid result.

### *S-series:*

- Following reconstitution, the S-series should be stored at -20°C or alternatively in a fridge (4-7°C) if no freezer is available until finished.

### *G-scores:*

- The G-scores should be stored immediately after use in a dark and dry place.

# 5. REFERENCES

Rapid Medical Diagnostics, Pretoria, South Africa – including references in the pamphlet. http://www.rapid-diagnostics.com/updates_22_11_2017/RMD_Pamphlet_10_05_17.pdf
Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

van Dam GJ, Wichers JH, Ferreira TM, Ghati D, van Amerongen A, Deelder AM: Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. Journal of clinical microbiology 2004, 42(12):5458-5461.

# APPENDIX 1. TEMPLATE FOR RECORDING RESULTS

**POC CCA –** Data collection sheet

| | |
|---|---|
| **Study details** | |
| **Date** | |
| **Name of technician** | |
| **POC-CCA batch number and expiration date** | |

| ID Participant | G-score |
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