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An innovative and user-friendly scoring system for standardised 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 (Spearman 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-scores for their field work. Further distribution of the cassettes, in particular when provided in combination with reference standards, will assist the wider schistosomiasis community in dealing with issues like batch-to-batch differences and interpretation of trace readings.

1. 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 (Van Dam et al., 2004). 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 (Bergquist 2013; Utzinger et al., 2015).

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 (Coulibaly et al., 2013; Dawson et al., 2013; Kittur et al., 2016). 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 (Colley et al., 2017).

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 with different test line intensities to allow scoring on a 1 to 10 scale (G1–G10), see Fig. 1. The scale attempts to mimic the range of colour intensity that can be observed, allowing less reader dependency.

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Fig. 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-scores.

2. 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 160 mg 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 (Tukahebwa et al., 2013), was used to demonstrate the use of the G-scores. 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 (Fig. 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 (see SOP in Supplementary file 1). 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) (Van Dam et al., 1994).

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+

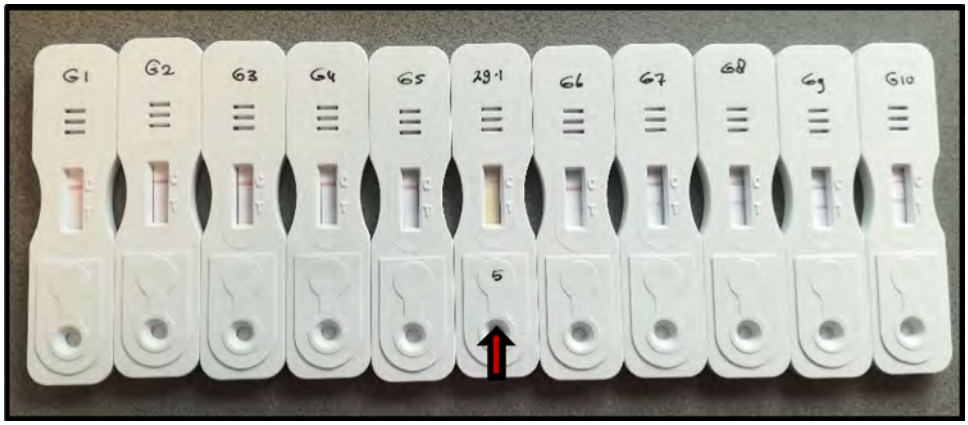

Fig. 2. Example of scoring a urine sample using the G-scores. The arrow indicates the cassette to be scored, which is placed in between the G-scores that resemble the intensity of the test line in order to select the best matching G-score.

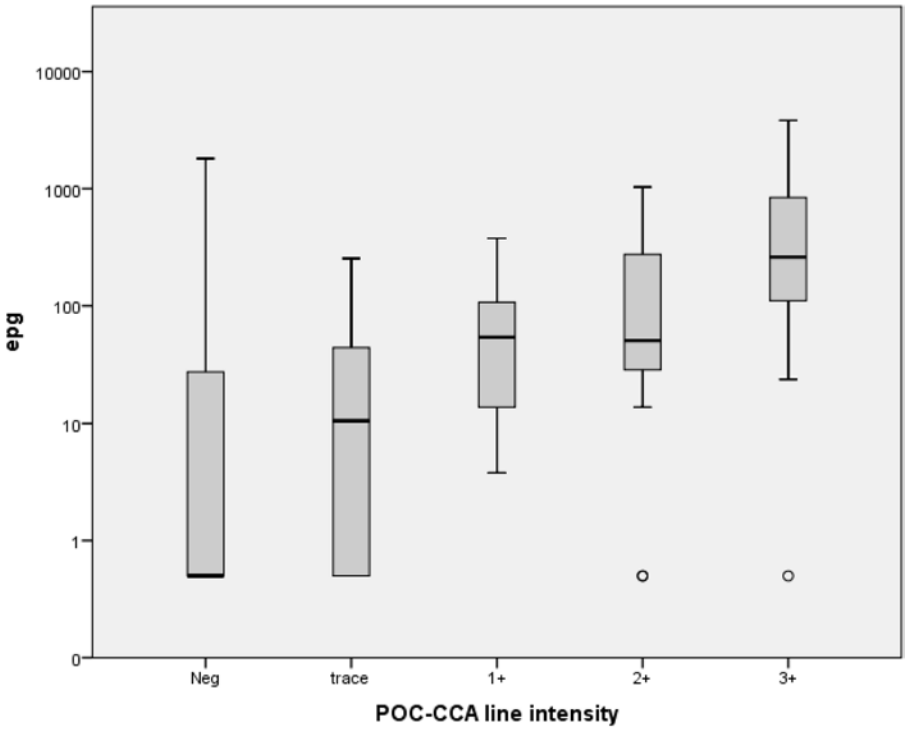

Fig. 3. Boxplot representing correlation between egg per gram faeces (epg) by Kato-Katz smear and line intensity by POC-CCA. For the purpose of log-transformation a value of 0.5 was added to all data. The 5 POC-CCA commonly used categories correspond to the converted G-scores using Table 1. A significant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

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) (Fig. 3) (Supplementary Table 1). 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 (Polman et al., 2000).

4. 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 in using these cassettes (Haggag et al., 2019). Based on these enthusiastic responses the G-scores, with the accompanying S-series and SOP, 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 as a standalone optical reader or a phone application specifically designed to assist the readouts.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. The POC-CCA test evaluated in this manuscript has originally been developed in our laboratory. The test is now licensed out. Still, the monoclonal antibody used for the test is still provided by our department and a revenue is received.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2019.105150.

References

Bergquist, R., 2013. Good things are worth waiting for. Am. J. Trop. Med. Hyg. 88 (3), 409–410.

Colley, D.G., Andros, T.S., Campbell Jr., C.H., 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.

Coulibaly, J.T., N'Gbesso, Y.K., Knopp, S., N'Guessan, N.A., Silue, K.D., van Dam, G.J., 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.

Dawson, E.M., Sousa-Figueiredo, J.C., Kabatereine, N.B., Doenhoff, M.J., Stothard, J.R., 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), 639–647.

Haggag, A.A., Casacuberta Partal, M., Rabiee, A., Abd Elaziz, K.M., Campbell, C.H., Colley, D.G., 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), 1507–1511.

Kittur, N., Castleman, J.D., Campbell, C.H., King, C.H., Colley, D.G., 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), 605–610.

Polman, K., Diakhate, M.M., Engels, D., Nahimana, S., Van Dam, G.J., Falcao Ferreira, S.T., 2000. Specificity of circulating antigen detection for Schistosomiasis mansoni in Senegal and Burundi. Trop. Med. Int. Health 5 (8), 534–537.

Tukahebwa, E.M., Magnussen, P., Madsen, H., Kabatereine, N.B., 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.

Utzinger, J., Becker, S.L., van Lieshout, L., van Dam, G.J., Knopp, S., 2015. New diagnostic tools in schistosomiasis. Clin. Microbiol. Infect. 21 (6), 529–542.

van Dam, G.J., Bergwerff, A.A., Thomas-Oates, J.E., Rotmans, J.P., Kamerling, J.P., Vliegenthart, J.F., 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), 467–482.

van Dam, G.J., Wichers, J.H., Ferreira, T.M., Ghati, D., van Amerongen, A., Deelder, A.M., 2004. Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. J. Clin. Microbiol. 42 (12), 5458–5461.