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Longevity of *Schistosoma mansoni* circulating cathodic antigens in filter paper dried urine spots

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ABSTRACT

Objectives: This study aims to determine the temporal stability of *Schistosoma mansoni* circulating cathodic antigens (CCA) in filter paper-based dried urine spot (FP-DUS) samples under varying temperatures condition.
Methods: Urine from 20 children confirmed to have *S. mansoni* infection using Kato-Katz (at least 1 egg per gram of stool) and Schisto POC-CCA (2+ and 3+) methods were stored in form of FP-DUS and urine at room temperature (RT), 4 °C and −20 °C. Standard urine and FP-DUS Schisto POC-CCA methods were employed to detect CCA in urine and FP-DUS samples respectively, at weeks 4, 8 and 12. The results were reported as negative or positive (trace, 1+. 2+, and 3+).
Results: In FP-DUS samples, POC-CCA scores initially increase after 4–8 weeks, but then showed a decrease in intensity while still remaining positive, independent of temperature condition. From week 4 to week 12, at least 80 % of urine samples had POC-CCA score of 3+, independent of temperature condition. However, 2 urine samples at RT tested negative at weeks 8 and 12.
Conclusions: Despite the decrease in the intensity of test line in many samples, *S. mansoni* CCA remains stable and detectable in urine samples stored in FP-DUS.

1. Introduction

Schistosomiasis, a neglected tropical disease caused by parasitic flatworms of the genus *Schistosoma*, remains a major global health concern [1]. Traditional diagnostic methods for schistosomiasis, such as microscopy, have limitations in sensitivity, especially in detecting persons with low-intensity infections [2]. The use of *Schistosoma* circulating antigens, specifically the point-of-care circulating cathodic antigen (POC-CCA) assays, has emerged as a promising alternative for its enhanced sensitivity and applicability to non-invasive urine-based testing [1,2]. The *Schistosoma* POC-CCA assays for determination of *Schistosoma mansoni* prevalence has recently been recommended in the current WHO guideline for use during programmatic surveys [1], however, the POC-CCA are sold commercially by single company [3]. The presence of single manufacturer impedes the timely availability of the test cassettes. Moreover, it may come a time as with the COVID-19 pandemic where industrial production become down and material transportation within and across continents become difficult. Furthermore, majority of schistosomiasis control programs rely on the support of their development partners' (donors) timeline to implement their activities [4]. Therefore, the program may require the use of low-cost

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sample collection and storage methods that could be used even in a resource-limited setting, especially when time for data (specimen) collection is an integral part of program activities [5]. In previous studies, we have reported the promising results of filter paper-based dried urine spot (FP-DUS) method for urine collection and detection of *S. mansoni* CCA in resource limited settings [4,5]. However, the temporal stability (longevity) of *S. mansoni* CCA in FP-DUS samples has not been fully investigated. Understanding the longevity of *S. mansoni* CCA in FP-DUS samples is critical to determining the diagnostic method's feasibility and reliability. The purpose of this study is to contribute to the optimization and validation of the DUS method for diagnosing schistosomiasis by evaluating the temporal stability of *S. mansoni* CCA in FP-DUS samples under varying temperatures.

2. Methods

2.1. Study design

This was laboratory based experimental study conducted between November 2022 and February 2023. The stored samples were from 20 SAC who were confirmed to be infected by *S. mansoni* using duplicate Kato-Katz smears (stool samples), standard urine Schisto POC-CCA method (urine samples) and FP-DUS Schisto POC-CCA method (dried urine samples) in our previous study [5]. The field and laboratory preparation of urine and FP-DUS samples were all described in our previous publication [5]. Fig. 1 illustrate the procedures performed on each urine sample. Briefly, FP-DUS were prepared by spotting urine from each SAC on three separate prelabeled 2 × 3 inch filter papers (Whatman protein saver). The remaining urine was aliquoted into three separate prelabeled Eppendorf tubes. The prepared FP-DUS samples and aliquoted urine samples were tested for *Schistosoma* CCA before storage (baseline or week 0), using standard urine and FP-DUS Schisto POC-CCA methods, respectively. Storage was done by distributing the three filter papers and their respective Eppendorf tubes to the three storage temperatures (room temperature (RT) at around 25 °C, 4 °C and −20 °C). Hence, each SAC had FP-DUS sample and urine sample stored in each of the three temperatures. The samples were stored over a period of 12 weeks and tested for *Schistosoma* CCA at week 4, 8 and 12 using the standard urine and FP-DUS Schisto POC-CCA methods for urine and FP-DUS samples respectively [5]. Rigorous quality control measures were implemented throughout the experiments including monitoring of refrigerator and freezer temperatures to ensure the integrity of the stored samples. The results of both standard urine and FP-DUS Schisto POC-CCA methods were reported as positive or negative. Positive results were further scored visually as trace, 1+. 2+,

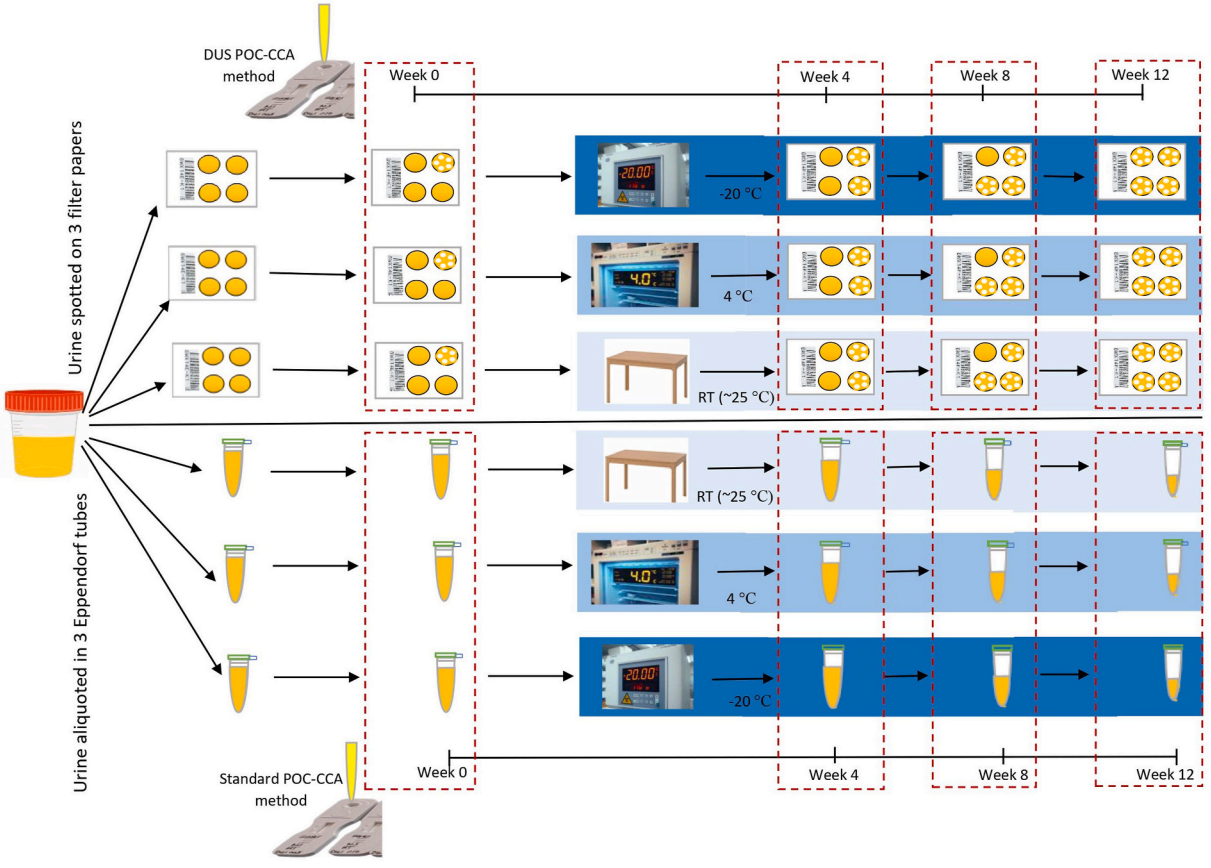

Fig. 1. Illustrate the procedures performed on each of the 20 urine samples.

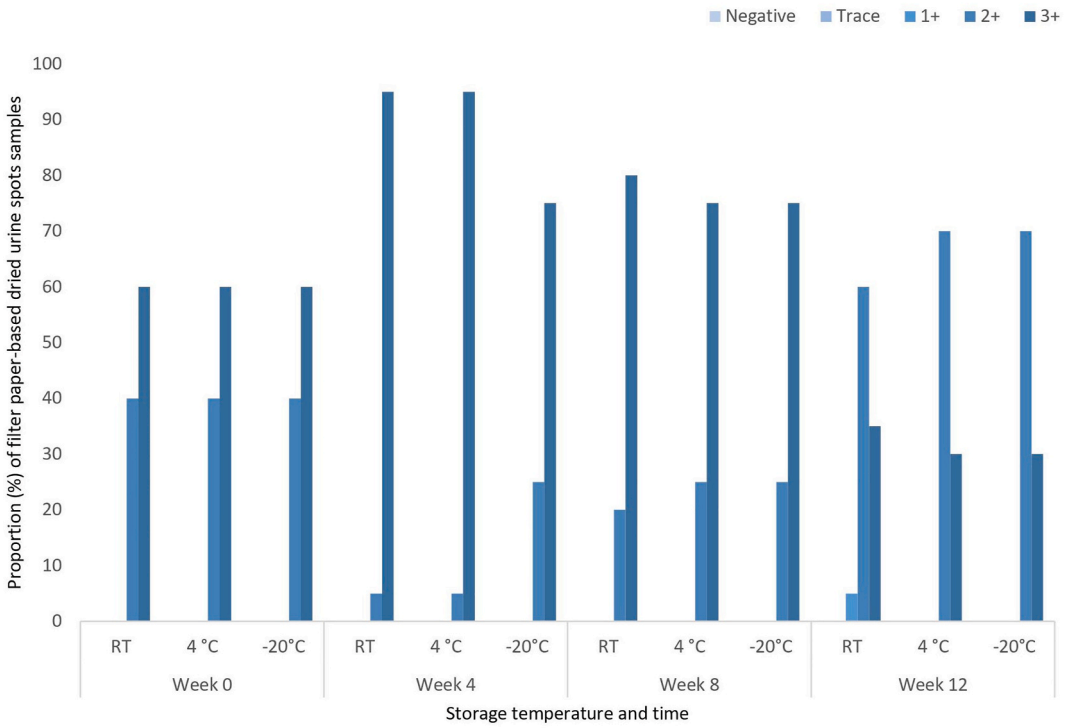

Fig. 2. Presents the visual score results based on the test line intensity of the 20 *S. mansoni* CCA positive samples spotted on filter paper and stored at different temperatures at various point of time period.

and 3+ based on the intensity of test line (see Fig. 2).

3. Results

3.1. Stored filter paper-based dried urine spot samples

The study involved stored urine and FP-DUS samples from 20 SAC who were confirmed to be infected with *S. mansoni* at baseline (week 0) based on KK (at least 1 egg per gram of stool) as well as on standard POC-CCA with a score ranging from 2+ to 3+, In the FP-DUS samples, POC-CCA scores initially increase after 4–8 weeks, but then showed a decrease in intensity while still remaining positive, independent of the temperature condition. The number of samples scored 3+ increased from 12 (60 %) to 19 (95 %) during week 4 for samples stored at RT and 4 °C, while the number of samples scored 3+ increased from 12 (60 %) to 15 (75 %) at −20 °C. During week 8, the number of samples with a score of 3+ decreased from 19 (95 %) to 16 (80 %) for RT and 15 (75 %) for 4 °C. Week 12 observed a further decrease in the number of samples with a 3+ score, with only 7 (53 %) samples reported for RT and 5 (25 %) samples for both 4 °C and −20 °C. Except for one sample that changed to 1+ score after 12 weeks of storage in RT, all samples that changed in their test line intensity were changed from 3+ to 2+.

3.2. Stored urine samples

In the urine samples, POC-CCA scores increased over time, independent of the temperature condition. During the first 4 weeks of storage, the number of samples with the test line intensity score of 3+ increased from 12 (60 %) to 16 (80 %) at RT and from 12 (60 %) to 20 (100 %) at 4 °C and −20 °C. With the exception of samples stored at RT, all other samples remained positive with test line intensity score of 3+ at week 8 and 12. One sample maintained its visual intensity of 2+ throughout storage time at RT. Three samples stored at RT showed the following changes: one sample changed from 2+ to 1+ at week 4, and from 1+ to negative at weeks 8 and 12. Two samples that scored 2+ in weeks 1 and 4 became negative (1 sample) and trace (1 sample) after 8 and 12 weeks (Fig. 3).

4. Discussion

The current study recorded the test line intensity score of the POC-CCA samples initially increase after 4–8 weeks, but then decreased while still remaining positive, independent of the temperature condition. Also, the study shows that *S. mansoni* CCA remains stable and detectable in urine samples stored at 4 °C and −20 °C for up to three months. The results are in accordance with the Schisto

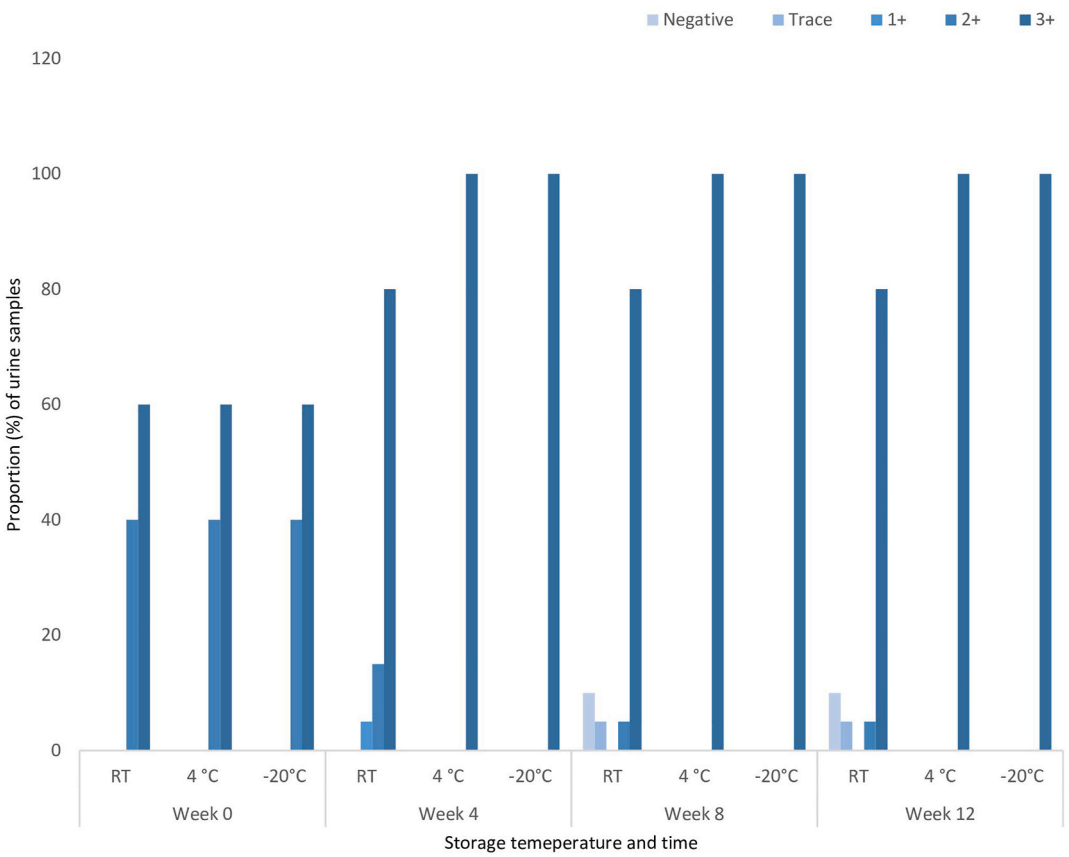

Fig. 3. Presents the visual score results based on the test line intensity of the 20 *S. mansoni* CCA positive urine samples stored at different temperatures at various point of time period.

POC-CCA cassettes manufacturer's instruction stated that urine for detection of *Schistosoma* CCA should be stored at 4 °C for at least 7 days or at −20 °C for at least 1 calendar year [6]. The decrease in the intensity of test line in DUS samples or change from positive to negative in urine samples could be due to other storage factors that are known to cause sample (analyte instability) degradation such as contact with air and light exposure [7]. None of the FP-DUS samples at RT changed to negative or trace for the entire storage time, while two and one urine samples turned to negative and trace respectively, after 12 weeks of storage at RT. This is a promising results as the storage method is intended for use in areas with limited resources. This study had some limitations, including the small sample size, the inability to detect the exact amount of *Schistosoma* CCA in both urine and FP-DUS, and the limited availability of Schisto POC-CCA cassettes, which forced us to limit our follow-up to 12 weeks.

5. Conclusion

Despite a decrease in the intensity of Schisto POC-CCA cassettes test lines in many samples, *S. mansoni* CCAs are stable and detectable in urine samples stored in FP-DUS. The FP-DUS sample storage method for urine samples is potential alternative to urine sample storage, particularly at RT. However, the method must be further refined before it can be recommended for use in routine and control program activities.

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CRediT authorship contribution statement

Abdallah Zacharia: Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Clemence Kinabo:** Writing – review & editing, Methodology, Data curation. **Twilumba Makene:** Writing – review & editing, Methodology, Data curation. **Huda Omary:** Writing – review & editing, Formal analysis. **George Ogweno:** Methodology, Data curation. **Faraja Lyamuya:** Methodology. **Billy Ngasala:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] World Health Organization, Guideline on Control and Elimination of Human Schistosomiasis, World Health Organization, Geneva, 2022, p. 142.
[2] P.T. Hoekstra, J. Madinga, P. Lutumba, R. van Grootveld, E.A.T. Brienen, P.L.A.M. Corstjens, et al., Diagnosis of schistosomiasis without a microscope: evaluating circulating antigen (CCA, CAA) and DNA detection methods on banked samples of a community-based survey from DR Congo, Trop Med Infect Dis 7 (10) (2022).
[3] H.L. Shane, J.R. Verani, B. Abudho, S.P. Montgomery, A.J. Blackstock, P.N.M. Mwinzi, et al., Evaluation of urine CCA assays for detection of *Schistosoma mansoni* infection in Western Kenya, PloS Negl Trop Dis 5 (1) (2011) 1–7, https://doi.org/10.1371/journal.pntd.0000951.
[4] A. Zacharia, T. Makene, C. Kinabo, G. Ogweno, F. Lyamuya, B. Ngasala, Dried urine spot method for detection of *Schistosoma mansoni* circulating cathodic antigen in resource-limited settings: a proof of concept study, Front. Immunol. (2023) 1216710, https://doi.org/10.3389/fimmu.2023.1216710, 14: 14.
[5] A. Zacharia, C. Kinabo, T. Makene, H. Omary, G. Ogweno, F. Lyamuya, et al., Accuracy and precision of dried urine spot method for the detection of *Schistosoma mansoni* circulating cathodic antigens in resource - limited settings, Infect Dis Poverty 13 (15) (2024) 1–10, https://doi.org/10.1186/s40249-024-01183-7.
[6] Rapid Medical Diagnostics, Schisto POC-CCA Rapid Test for Qualitative Detection of Bilharzia (Schistosomiasis), 2018.
[7] R.G. Rioja, D.M. Espartosa, M. Segovia, M. Ibarz, M.A. Llopis, J.M. Bauca, et al., Laboratory sample stability. Is it possible to define a consensus stability function? An example of five blood magnitudes, Clin. Chem. Lab. Med. 56 (11) (2018) 1806–1818.