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Low specificity of point-of-care circulating cathodic antigen (POC—CCA) diagnostic test in a non-endemic area for schistosomiasis mansoni in Brazil

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ABSTRACT

A point-of-care test for detecting schistosome circulating cathodic antigen in urine (POC—CCA) has been proposed for mapping infection and defining prevalence thresholds for mass drug administration (MDA). However, there is increasing evidence that POC—CCA may yield false-positive results, which requires rigorous specificity evaluation in non-endemic areas. POC—CCA was applied in an area known to be free from infection and devoid of any condition for schistosomiasis transmission as part of a multicentre study to evaluate the performance of POC—CCA in Brazil's low or potentially endemic settings. Besides POC—CCA detection in urine, a search for eggs in stool was performed by Kato-Katz (KK) and Helmintex (HTX) methods. One-hundred-and-seventy-four participants returned urine samples, 140 of which delivered stool samples. All these were HTX-negative for *Schistosoma mansoni*, and all 118 tested with KK were negative for both *S. mansoni* and soil-transmitted helminths. POC—CCA results from freshly collected urine yielded a specificity of 62.1% (95% CI: 53.6% - 70.2%), taking trace outcomes as positive according to the manufacturer's instructions. Retesting urine from the 140 HTX-negatives after one-year storage at -20 °C with two new POC—CCA batches simultaneously yielded significantly different specificities (34.3%; 95%CI: 26.5% – 42.8% and 75.0%; 95% CI: 67.0% - 81.9%). These two batches had a weak agreement (Cohen's kappa: 0.56; 95%CI: 0.44–0.68) among the 174 urine samples retested. At present, POC—CCA cannot be recommended either as a cut-off point for MDA or a reliable diagnostic tool for treatment of the infection carriers (selective chemotherapy) in low endemic areas and at final stages of transmission interruption. Manufacturers should be required to optimize production standardization and to assure quality and reproducibility of the test. Extended rigorous performance evaluations by different users from different regions are needed before POC—CCA is widely recommended.

1. Introduction

Schistosomiasis is a highly prevalent helminthiasis among the poorest populations, caused by infection with trematode worms of the genus *Schistosoma*. According to The Global Burden of Diseases, Injuries, and Risk Factors Study 2017 (GDB-2017 2018), an estimated 143.8

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million (95% Uncertainty Interval [UI]: 131.7 – 155.5 million) people are affected, mainly in Africa. In the Americas, where the only etiological agent is *Schistosoma mansoni*, Brazil accounts for 95% of the infections (WHO 2010). The most recent national prevalence survey in the country estimates that 1% of the population of 209 million may be infected (Katz 2018).

In 2012, endemic countries at the World Health Assembly adopted Resolution WHA65.21 with the commitment to intensify the control of schistosomiasis aiming its elimination as a public health problem (WHO 2012a). In compliance with this Resolution, the Brazilian Ministry of Health (MoH) formulated an Action Plan aiming to reduce the prevalence of infection, the occurrence of severe forms and deaths and the risk of disease expansion, through early diagnosis, timely treatment with praziquantel and surveillance as well as judicious control of intermediate hosts, appropriate environmental sanitation and effective health education (Brasil 2014). For endemic areas, the MoH prioritizes periodic parasitological surveys of whole at-risk communities in municipalities with egg-positivity $\geq$ 5% with a single sample with two slides of Kato-Katz (KK) fecal thick smear (Katz et al., 1972), followed either by treatment of the infection carriers (selective chemotherapy, as characterized by Gabrielli et al., 2011) or mass drug administration (MDA) according to the prevalence class (Favre et al., 2015).

In 2019, the MoH established a four-year intensification of the Action Plan in which 472 priority municipalities have agreed to perform a community-wide baseline survey of at-risk localities and carry out the following treatment scheme: biennial, community-wide MDA if KK egg-positivity (one sample, two slides) is $\geq$ 10% (high risk), or treatment of the positives if $<$ 10% (low risk). Since the endemic areas of Brazil are considered to be low risk (WHO 2012b), in most localities the treatment scheme will require the identification of the infection carriers (selective chemotherapy).

A point-of-care immunochromatographic test for detection of schistosome circulating cathodic antigen in urine (POC—CCA) has been proposed as a rapid, sensitive alternative to KK for mapping infection as well as defining prevalence thresholds for several treatment schemes (Bärenbold et al. 2018), which has been recently endorsed by WHO (https://www.who.int/activities/enhancing-implementation-of-schistosomiasis-control-and-elimination-programmes Accessed 28/5/2020). Several studies have presented estimated high sensitivities when compared to egg-detection methods (Danso-Appiah et al., 2016). Antigen-positive outcomes have been reported even in individuals tested negative with a combination of exhaustive, rigorous diagnostic methods based on egg detection from higher amounts of fecal material or increased sampling effort (Ferreira et al., 2017; Oliveira et al., 2018; Grenfell et al., 2019; Haggag et al., 2019a; Haggag et al., 2019b, Magalhães et al., 2020; Souza et al., 2020). It has been argued that these outcomes result from failure to detect eggs when oviposition is greatly decreased or suppressed (Colley et al., 2017; Armoo et al., 2020). However, there are also indications that POC—CCA may yield false-positive results (Coelho et al., 2016; Colley et al., 2017; Bezerra et al., 2018; Haggag et al., 2019a; Haggag et al., 2019b). A rigorous evaluation of specificity requires testing populations known to be free of infection, where POC—CCA is expected to yield only negative outcomes (Danso-Appiah et al., 2016; Peralta and Cavalcanti 2018). Here we present data on antigen detection in urine with POC—CCA in a non-endemic locality in the southernmost Brazilian State, Rio Grande do Sul (RGS). The data set contributes to better estimate true-negative (specificity) and false-positive rates. The investigation is part of an ongoing multicentre study commissioned by the Ministry of Health (MoH) to access the performance of POC—CCA for community interventions in Brazil.

2. Material and methods

2.1. Ethics statement

The multicentre study protocol was approved by the Ethics Committee of Oswaldo Cruz Institute (CEP-IOC) - Fiocruz (CAAE: 82,469,417.8.0000.5248). The study followed the guidelines and regulations for research involving human beings (Resolution 196/1996 of the National Health Council) and is in accordance with the principles of the Declaration of Helsinki, contained in section II, Article 15.1. Potential subjects were informed about the research objectives, procedures, risks, possible discomforts, benefits, duration, as well as their freedom to leave at any time of the study. The research team ensured that potential subjects understood all information and that their consent was voluntary. The study was conducted according to the approved protocol, Good Clinical Practices (GCP) and the applicable regulatory requirements.

2.2. Study area and subject sampling

Marau (28° 26′ S; 52° 12′ W) is located at the highlands (altitude 571 m) and it is 265 km from Porto Alegre, the capital city of RGS. RGS is a schistosomiasis non-endemic area (Brasil 2014; Katz, 2018), except for the municipality of Esteio, in the Porto Alegre metropolitan area and 245 km from Marau. In 1997, a focal transmission site was detected in Esteio, but only 28 infected individuals were found in two decades. Several surveys demonstrated ultra-low positivity rates (below 0.13%) and a last documented infection was registered in 2011 (Ramírez et al., 2020).

The nearest active transmission area is 800 Km to the north of Marau, in the State of Paraná. Marau has an estimated number of 44,161 inhabitants, mostly of Italian origin, with a averaged monthly income of US$ 453, a gross domestic product (GDP) *per capita* of US$ 5579 (1 US$ = *R*$ 5.53, 23/3/2020) and human development index (HDI) of 0.773, ranking 185 among the 5570 Brazilian municipalities. Adequate sewage collection and treatment serves 87.2% of the households, ranking 594 in Brazil and 45 in RGS; 83.3% of households are served by treated water (https://cidades.ibge.gov.br/brasil/rs/marau/panorama; https://www.br.undp.org/content/brazil/pt/home/idh0/rankings/idhm-municipios-2010.html Accessed 28/5/2020). It was chosen as the non-endemic area for the study because of its good sanitary and socioeconomic situation and the absence of any of the conditions for schistosomiasis transmission.

The minimum sample size was calculated as 140 for a specificity of 90% (false-positive rate: 10%), a confidence interval of 95% (CI 95%) and a precision of 0.05 (Zaidi et al., 2016). Sampling was not meant to represent the whole population. For operational reasons, the recruitment of individuals and initial processing of samples was centered at a municipal school, *Escola Municipal Frei Wilson João Sperandio*. Educational activities with students were followed by meetings with the families and all were invited to participate in the study. However, care was taken not to recruit anyone with any history of travel to or any past residence in endemic areas. The families were living next to the School, in the central urbanized area of Marau, without lakes, rivers or other water bodies that could favor the existence of snail breeding sites.

All residents older than two years of age were eligible to participate in the study. However, pregnancy, self-reported or clinical signs/symptoms of acute illness and chronic severe diseases were exclusion criteria. For operational reasons participants were recruited and tested in two runs: July and September 2019. Upon signing and/or orally assenting to the Free and Informed Consent Form, each participant

received a vial to collect one sample of 10 ml midstream urine to be returned on the day of the visit, and a container to collect one sample of 50 g of stool to be returned up to the following day. Urine and stool samples were code-identified, stored in polystyrene cooler boxes, and taken to the laboratory.

2.3. Sample processing and testing

The freshly collected urine samples were tested with POC—CCA (TR.0301CA020, batch number: 180907091; expiry date: 30 August 2020) according to the manufacturer's instructions (ECO Diagnóstica Ltda., Brazil -Reg MoH 80954880012, Ed. 001/2018, approved on 12/06/2018). Transportation and storage of the cassettes until use also followed strictly the manufacturer's recommendations. A minimum of 5 ml of each urine sample was stored at −20 °C to check its stability for testing after at least one year.

The POC—CCA outcome, read in the 21st minute, was scored as described by Casacuberta-Portal et al. (2019), with reference cassettes kindly provided by Dr Govert J. van Dam, Leiden University Medical Centre. The required amount of stool (50 g) was intended for both KK and Helmintex (HTX); the former is largely used in routine control programs, including in Brazil, and the latter is a highly sensitive egg-detection test proposed as a reference method (Lindholz et al., 2018). For the KK, 84 mg of stool (two slides per sample) were prepared and read as described by Katz et al. (1972). While eggs from most soil-transmitted helminths (STH) cannot be reliably detected with HTX, they were searched in KK preparations. A large volume of stool (30 g) was processed for HTX method and examination at the microscope was performed according to Favero et al. (2017) and De Souza et al. (2019). All KK slides and HTX preparations from this study were totally screened under the microscope by the first author (C.Graeff-Teixeira) with large experience in identification *of S. mansoni* eggs and a rigorous operational protocol. The protocol involves regular breaks to avoid "boring effect" and failure to keep attention at the examination, especially because the large number of negative samples. It also involves the clear criteria for identification of eggs (De Souza et al. 2019). KK and HTX results were taken together to confirm egg-negative status in the studied population.

All participants were also tested with strips for semiquantitative determination of the following parameters in urine: Leukocytes, Urobilinogen, Bilirubin, Occult Blood, Nitrites, Ph, Specific Density, Protein, Glucose and Ketones (Cral Sensi 10 – Cral Artigos para Laboratório Ltda, Cotia, SP Brazil https://www.cralplast.com.br/produto/tira-para-uroanalise/ Accessed 28/5/2020)

At reviewer's recommendation, the urine samples stored at −20 °C were retested in September 2020 with two new batches of POC—CCA, one Eco Diagnóstica (ED), Brazil (batch: 202003014; valid until 31 January 2022) and other from Rapid Medical Diagnostics (RMD), South Africa (batch: 191031120; valid until 30 November 2021). The samples were thawed, homogenized, and tested at room temperature (25 °C). Each cassette was read out by three experienced technicians blinded to the results obtained in the freshly collected samples as well as to the batch used, and the G-score outcome was determined consensually.

2.4. Data presentation and analysis

The data were entered in an Excel spreadsheet and, after being crosschecked with the source documents, were transferred to Systat (https://systatsoftware.com/products/systat/ Accessed 28/5/2020) for statistical analysis. The POC—CCA outcomes were presented in G-scores and their corresponding visual scores, as follows: G1, negative; G2 and G3, trace; positive, from G4 to G10 (Casacuberta-Portal et al., 2019). The POC—CCA data were analysed considering traces (G2–G3) as positive (*t+*) as well as negative (*t-*). The results were expressed in absolute numbers and percentages with 95% confidence intervals (CI); non-overlapping 95% CI indicated significant differences ($p < 0.05$) between proportions. False-positive rate was calculated as the proportion of POC—CCA positives in relation to all individuals tested (confirmed as egg-negative). True-negative rate (specificity) was calculated as the proportion of POC—CCA negatives in relation to the tested individuals, using the MedCalc diagnostic test calculator (https://www.medcalc.org/calc/diagnostic_test.php Accessed 28/5/2020). As the two POC—CCA retesting batches were used for the same urine samples and under the same conditions, the agreement between them was estimated by Cohen's kappa (κ) statistic; a κ-value below 0.60 indicated inadequate agreement (McHugh, 2012). POC—CCA outcomes from both freshly collected and stored urine were compared by age-group (5–18 yrs / > 18 yrs), considering traces both as positive (*t+*) and negative (*t-*). Significant differences between the age groups were evaluated by the chi-square (χ^2) test, with *p*-values < 0.05 indicating significant difference

3. Results and discussion

A total of 174 (97.8%) out of 178 recruited residents provided urine samples, whereas 140 (78.7%) returned samples of both urine and stool. Of the 174 participants who provided urine samples, 85 (58.0%) were females. The youngest participant was five years old and the oldest, 82 years old; 64 (38.6%) participants were up to 18 years of age, averaging 9.7 (standard deviation: ±2.1) years. The remaining 110 (61.4%) were over 18 years old, averaging 41.3 (standard deviation: ±14.3) years (see Excel file in the Supplementary Material). The 140 fully complying participants were tested with HTX and 118 (84.3%) of them, with KK. As 22 individuals provided less than 30 g of stool, a decision was made to use all the fecal sample for the Helmintex method, which is 3–4 times more sensitive than Kato-Katz (Lindholz et al., 2018). This is the reason for a lower number of KK examinations.

All fully complying participants were confirmed as HTX negative for *S. mansoni*, and all 118 tested with KK were negative for both *S. mansoni* and STH (see Excel file in the Supplementary Material). A high proportion (62.1%; 95%CI: 52.1% – 71.0%) of them tested negative(G1) in the freshly collected urine with POC—CCA batch 180907091 from ED (ED1). However, trace (G2-G3) and positive (G4-G5) outcomes were detected respectively in 27.9% and 10.0% of the samples, resulting in a false-positive rate of 37.9%. The combination of trace and positive results lead to an estimated specificity of 62.1% (95% CI: 53.6% - 70.2%). Of the 14 subjects with non-trace positive outcomes (G4 or G5), nine were between 8 and 11 years of age, with families reporting that their children had never been in an endemic area for schistosomiasis; the remaining five, a 17-year-old teenager, a 30-year-old adult and three elderly women, denied any contact with endemic areas. Stored urine from the 140 fully complying participants yielded a specificity of 34.3% (95%CI: 26.5% – 42.8%) with POC—CCA batch 202003014 from ED (ED2) and 75.0% (95% CI: 67.0% - 81.9%) with batch 191031120 from RMD.

Results of three participants can be highlighted: two women (aged 22 and 37 years-old) and an eight-year-old boy, without abnormal parameters in the urine, tested POC—CCA negative (G1) with ED1 from freshly collected urine and yielded strong positive outcomes (G7 with ED2 and G6 with RMD) from stored urine. The Graphical Abstract shows an image of the cassettes from the 37-year-old woman (ID 008) who reiterated, after the end of the study, never traveled to an endemic area, and reported having made a single and brief trip to the city of Cuiabá, outside the endemic area of schistosomiasis.

Table 1 shows the POC—CCA results from all 174 participants who provided urine samples for testing. In freshly collected urine, where POC—CCA batch ED1 was used, outcome based on the G-score varied from G1 (63%) to G5 (2.3%); the maximum visual score was 1+ ("weak positive"); trace-positive (*t+*) scoring yielded 36.8% positives, whereas trace-negative (*t-*) scoring yielded 9.2% positives. In the stored urine, where batches ED2 and RMD were used, the G-score varied from G1 (33.9% with batch ED2 and 46.0% with batch RMD) to G10 (1.7% with ED2 and 1.1% with RMD); the visual score reached 3+ ("very strong positive") both with ED2 (24.0%) and RMD (2.9%). Trace-positive (*t+*)

Table 1
POC—CCA results from all 174 participants who provided urine samples from a non-endemic area of schistosomiasis mansoni in Brazil. G-scores and visual scores are presented according to Casacuberta-Portal et al. 2019; trace-positive scores and trace-negative scores are given considering traces (G2–G3) as positive (*t*+) and negative (*t*-), respectively. Freshly collected urine was tested with batch 180907091 from Eco Diagnóstica, Brazil, and retested simultaneously with batch 202003014 (also from Eco Diagnóstica) and batch 191031120 (from Rapid Medical Diagnostics, South Africa) after being stored at −20 °C for one year. N, number of cases; CI, confidence interval.

Freshly collected urine, tested by POC—CCA batch 180907091 from Eco Diagnostica, Brazil											
G-scoring			**Visual scoring**			**Trace-positive (*t*+) scoring**			**Trace-negative(*t*-) scoring**		
Outcome	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**
G1	110	63.2 (52.9, 72.2)	Negative	110	63.2 (53.7, 71.6)	Negative	110	63.2 (54.3, 71.2)	Negative	158	90.8 (84.4, 95.0)
G2	28	16.1 (9.4, 24.2)	Trace	48	27.6 (19.6, 36.3)	Positive	64	36.8 (28.5, 45.3)			
G3	20	11.5 (5.9, 18.9)									
G4	12	6.9 (2.7, 13.2)	1+	16	9.2 (4.5, 15.6)				Positive	16	9.2 (4.8, 15.2)
G5	4	2.3 (0.3, 6.8)									
TOTAL	174	100.0		174	100.0		174	100.0		174	100.0
Urine stored at −20 °C for one year, retested by POC—CCA batch 202003014 from Eco Diagnóstica, Brazil											
G-scoring			**Visual scoring**			**Trace-positive (*t*+) scoring**			**Trace-negative(*t*-) scoring**		
Outcome	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**
G1	59	33.9 (23.9, 44.3)	Negative	59	33.9 (24.7, 43.5)	Negative	59	33.9 (25.8, 42.4)	Negative	135	77.6 (69.4, 84.1)
G2	39	22.4 (14.0, 32.1)	Trace	76	43.7 (33.7, 53.4)	Positive	115	66.1 (57.3, 73.8)			
G3	37	21.3 (13.0, 30.8)									
G4	14	8.0 (3.2, 15.2)	1+	22	12.6 (6.8, 20.2)				Positive	39	22.4 (15.6, 30.2)
G5	8	4.6 (1.1, 10.7)									
G6	3	1.7 (0.0, 6.4)	2+	10	5.7 (2.0, 11.7)						
G7	7	4.0 (0.9, 9.8)									
G8	1	0.6 (0.0, 4.3)	3+	7	4.0 (1.0, 9.3)						
G9	3	1.7 (0.0, 6.4)									
G10	3	1.7 (0.0, 6.4)									
TOTAL	174	100.0		174	100.0		174	100.0		174	100.0
Urine stored at −20 °C for one year, retested by POC—CCA batch 191031120 from Rapid Medical Diagnostics, South Africa											
G-scoring			**Visual scoring**			**Trace-positive (*t*+) scoring**			**Trace-negative(*t*-) scoring**		
Outcome	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**	**Outcome**	**N**	**% (95% CI)**
G1	80	46.0 (35.0, 56.5)	Negative	79	45.4 (35.3, 55.2)	Negative	80	46.0 (37.2, 54.6)	Negative	150	86.2 (79.0, 91.4)
G2	53	30.5 (20.8, 40.7)	Trace	71	40.8 (31.0, 50.5)	Positive	94	54.0 (45.1, 62.4)			
G3	17	9.8 (4.3, 17.4)									
G4	5	2.9 (0.4, 8.2)	1+	9	5.2 (1.7, 10.9)				Positive	24	13.7 (8.4, 20.6)
G5	4	2.3 (0.2, 7.3)									
G6	4	2.3 (0.2, 7.3)	2+	10	5.7 (2.0, 11.7)						
G7	6	3.4 (0.6, 9.0)									
G8	1	0.6 (0.0, 4.3)	3+	5	2.9 (0.5, 7.7)						
G9	2	1.1 (0.0, 5.4)									
G10	2	1.1 (0.0, 5.4)									
TOTAL	174	100.0		174	100.0		174	100.0		174	100.0

scoring yielded 66.1% positives with ED2 and 54.0% positives with RMD, whereas trace-negative (*t*-) scoring yielded 22.4% with ED2 and 13.7% with RMD. As indicated by the non-overlapping 95%CIs of the trace-positive (*t*+) outcomes, the proportion of positives in freshly collected urine (36.8% with ED1) was significantly lower than in stored urine (66.1% with ED2 and 54.0% with RMD); as regards trace-negative (*t*-) outcomes, the proportion of positives in freshly collected urine (9.2%with ED1) was significantly lower than in stored urine tested with ED-2 (22.4%) but not with RMD (13.7%). Agreement between the two POC—CCA batches (ED-2 and RMD) used in the stored urine was acceptable (Cohen's kappa $\geq$ 0.60) when traces were considered as negative (*t*-) (κ-value = 0.68; 95%CI: 0.54–0.82) but not when traces were considered as positive (*t*+) (κ-value = 0.56; 95%CI: 0.44–0.68).

Table 2 shows the outcomes by age group (5 - 18 yrs. / > 18 yrs.) among the 174 participants tested with POC—CCA from both freshly collected and stored urine, 64 in the youngest group and 110 in the oldest group. Positive outcomes from freshly collected urine occurred significantly more (p-value < 0.05) in the youngest group than in the oldest group, as assayed with batch 180,907,091, irrespectively of considering trace as positive (*t*+) or as negative (*t*-). In contrast, positive outcomes from stored urine, as assayed both with batch ED2 and RMD, occurred significantly less (p-value < 0.05) in the youngest group considering trace as positive (*t*+);there was no significant difference between the age groups (p-value > 0.05) with either batches considering trace as negative (*t*-). Agreement between the two POC—CCA batches from stored urine was acceptable in the oldest age group (κ-value = 0.62; 95%CI: 0.48–0.76) but not in the youngest age group (κ-value = 0.41; 95%CI: 0.18–0.64) when trace was considered as positive (*t*+). Agreement between the batches was acceptable both in the oldest (κ-value = 0.63; 95%CI: 0.45–0.81) and in the youngest (κ-value = 0.77; 95%CI: 0.55–0.98) group when trace was considered as negative (*t*-).

Considering that reiterated cross-questioning of the examined population in Marau failed to support the hypothesis of missed schistosomiasis infections, explanations for such unspecific POC—CCA reactions are so far unknown and intriguing, asking for further investigations. The other well demonstrated main limitation of the test is its lack of reproducibility when several batches were compared.

Another issue requiring further investigation is the manufacturer's claim that urine samples can be stored at −20 °C for one year before testing, as the significant discrepancies between freshly collected and stored urine found here cannot be ascribed exclusively to differences in batch performance. As a part of the multicentre study, an investigation is under way to evaluate the reliability of POC—CCA assay for diagnosing schistosomiasis mansoni in urine stored at −20 °C after one year by comparing its outcome with that of freshly collected urine from the same individuals in an Brazilian endemic area.

It is of interest that 25 (49.0%; 95% CI: 32.3% – 64.7%) out of 51 school-aged children (6 – 15 yrs.) assayed both with POC—CCA from freshly collected urine and HTX from stool were false positive (see Excel file in the Supplementary Material), giving a specificity of 51.0% (95% CI: 36.6% – 65.3%). These results are in contrast with those from a non-endemic area in Tanzania chosen to evaluate the original POC—CCA (Van Dam et al. 2004), where the specificity in school pupils ranging from 7 to 18 years of age was 86.7% (95% CI: 73.2% - 95.0%).

Table 2
POC—CCA outcomes by age group in Marau residents, a schistosomiasis non-endemic area in Brazil. The youngest group (5–18 yrs.) had 64 participants and the oldest (> 18yrs.), 110. Trace-positive outcomes and trace-negative outcomes are given considering traces as positive (*t*+) and negative (*t*-), respectively. Freshly collected urine was tested with batch 180907091 (from Eco Diagnóstica, Brazil), and retested simultaneously with batch 202003014 (also from Eco Diagnóstica) and batch 191031120 (from Rapid Medical Diagnostics, South Africa) after being stored at −20 °C for one year N. (%), number and percentages (in parentheses) of cases; CI, 95% confidence interval; $\chi 2$, chi-square value. *p*-value < 0.05 indicates a significant difference between age groups.

POC—CCA Assay Urine samples	Origin	Batch	Age group in years	Trace-positive (*t*+) Outcome	N (%)	Test statistics	Trace-negative(*t*-) Outcome	N (%)	Test statistics
Freshly collected	Eco Diagnóstica, Brazil	180907091	**5 – 18**	Positive	31 (48.4)	χ^2= 5.92 *p* = 0.015	Positive	12 (18.7)	χ^2= 11.07 *p* = 0.001
				Negative	33 (51.6)		Negative	52 (81.3)	
			> 18	Positive	33 (30.0)		Positive	4 (3.6)	
				Negative	77 (70.0)		Negative	106 (96.4)	
After one-year storage at −20 °C	Eco Diagnóstica, Brazil	202003014	**5 – 18**	Positive	49 (76.6)	χ^2= 4.96 *p* = 0.026	Positive	12 (18.7)	χ^2= 0.78 *p* = 0.377
				Negative	15 (23.4)		Negative	52 (81.3)	
			> 18	Positive	66 (60.0)		Positive	27 (24.5)	
				Negative	44 (40.0)		Negative	83 (75.5)	
	Rapid Medical Diagnostics, South Africa	191031120	**5 – 18**	Positive	41 (64.1)	χ^2= 4.11 *p* = 0.043	Positive	8 (12.5)	χ^2= 0.142 *p* = 0.706
				Negative	23 (35.9)		Negative	56 (87.5)	
			> 18	Positive	53 (48.2)		Positive	16 (14.5)	
				Negative	57 (51.8)		Negative	94 (85.5)	

Specificities of 99.0% (95%CI: 94.6%– 100.0%) and 100% (95% CI: 97.5% – 100.0%) were obtained from children (6 −16 yrs.) in non-endemic areas of Ethiopia (Colley et al., 2013) and Ecuador (Mwinzi et al., 2015), respectively, many of whom had STHs as detected with KK (Colley et al., 2017).

Our results, obtained with three different batches from both freshly collected and stored urine, show that POC—CCA cannot be regarded as a reliable indicator of prevalence threshold for the Americas, as proposed by Bärenbold et al. (2018). Based on the present results, the prevalence threshold of 10% by KK (one sample, two slides) is not translatable to 30% by POC—CCA and, therefore, should not serve as a cut-off point for the MoH Action Plan to recommend MDA in low or non-endemic localities. In fact, it would be advisable to assume 38% of false-positive outcomes as obtained with batch ED1 from freshly collected urine, and up to 66% of false-positive outcomes as obtained with batch ED2 from stored urine, considering the obtained specificity of 62% and 34%, respectively.

Failure of KK to detect *S. mansoni* egg-positive individuals in areas of low prevalence and/or low infection intensity disallows its use as a reference diagnostic method to evaluate POC—CCA performance in such endemic settings; as an alternative, HTX has been adopted as a highly sensitive egg-detection reference method (Lindholz et al., 2018, Oliveira et al. 2028, Magalhães et al., 2020). From the supplementary dataset provided by Lindholz et al., 2018 (https://doi.org/10.1371/journal.pntd.0006274.s002) it can be shown that, considering only subjects with no eggs or very low egg-load (epg <1) identified with HTX, POC—CCA performs unsatisfactorily. Thus, specificity of POC—CCA is 35.7% (95% CI: 30.0% - 41.8%) if trace is considered positive (*t*+) and sensitivity is 45.1% (95% CI: 36.1% - 54.4%) if trace is considered negative (*t*-); sensitivity of POC—CCA is further reduced to 12.5% (95% CI: 7.3% - 19.5%) if both trace and 1+ visual scorings are considered negative, in which case it does not differ significantly from that of KK (16.92%; 95% CI: 10.92% to 24.49%). As pointed out by Haggag et al. (2019b), POC—CCA trace and 1+ outcomes are not consistently altered after repeated treatments and may be regarded as false positives; that being the case, POC—CCA may well serve global schistosomiasis control analysis but not for areas of no or very low egg load. It is noteworthy that prevalence estimates based on KK and POC—CCA examinations can be similar if trace outcomes are considered as negative results (Armoo et al., 2020) and therefore could be of use as a preliminary tool in routine surveillance activities.

Significant variability in sensitivity and specificity among different versions (with buffer or without buffer) and batches from a same version of POC—CCA have been reported in Brazil (Viana et al. 2019; Grenfell et al., 2019), which may be ascribed to component, assembly or quality-control issues (Colley et al., 2020). However, even if lack of reproducibility is solved, the magnitude of false-positive reactions in non-endemic areas remains a challenging problem to be further investigated. One key action is establishing a collaborative multicentre well-characterized urine bank from diverse localities and populations.

One possible explanation for the POC—CCA not being sufficiently specific is cross reactivity of the antibody in the test and antigens related to pregnancy, other infections, neoplasia, and autoimmune diseases (Colley et al., 2017). In our study pregnancy, acute illness and chronic severe diseases were exclusion criteria, and no STH was detected by KK. Also, occurrence of abnormal parameters in urine had no significant effect in the POC—CCA outcome with the exception of leukocytes, which was significantly less likely to occur in POC—CCA positive samples (OR: 0.41; 95% CI: 0.18 – 0.93) (see Excel file in the Supplementary Material). Therefore, it is unlikely that the false-positive outcomes were due to such externalities.

In conclusion, the indicated limitations of specificity and reproducibility with POC—CCA prevents an unrestricted recommendation for its application not only as a cut-off point for MDA scheme but also as a reliable diagnostic tool for selective chemotherapy in low endemic areas and at final stages of transmission interruption. Manufacturers should be required to optimize production standardization and to assure quality and reproducibility of the test. Extended rigorous performance evaluations in different regions and by different users are necessary before POC—CCA is recommended for its global application as one reliable diagnostic tool for schistosomiasis control. As pointed out by Silva-Moraes et al. (2019) and Ferrer et al. (2020), in settings with low

prevalence and intensity of infection, a sequential and combinatory set of diagnostic tools will be needed for monitoring and for the final certification of absent schistosomiasis transmission.

4. Author contributions

Conceptualization, Validation, Methodology: CGT, FSMB,PMZC, MJE, TCF, NK, RRO, MGR, OSP; Writing – original draft: CGT, OSP; Writing – review & editing: CGT,PMZC, TCF, NK, MGR, OSP; Formal analysis, Visualization, Supervision: CGT, OSP; Investigation, Data curation: CGT, VF, VFP, RPS, FVR, LHD.

5. Author statement

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Declaration of Competing Interest

The authors declare that they have no competing interests

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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.2021.105863.

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