Journal of Immunological Methods 472 (2019) 1–6

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Suitability of commercially available POC-CCA tests for schistosomiasis: Considerations for efficiency, reproducibility and decision making criteria for field application in areas of low endemicity

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ARTICLE INFO

Keywords:
Schistosoma mansoni
POC-CCA®
Urine CCA (Schisto) ECO Teste®
POC FLT
Comparison between Kato-Katz and POC tests

ABSTRACT

Background: Point of care tests would be valuable for field diagnosis. However, high sensitivity will likely be required in low endemicity sets where individuals with low schistosome burden are hard to diagnose.
Methods: Commercially available POC tests (POC-CCA® and Urine CCA (Schisto) ECO Teste®) were evaluated to evidence their potential in low endemicity areas. Individuals with 0–76 eggs per gram of feces were selected, and comparison was performed between Kato-Katz, Saline Gradient and POC-CCA® after urine concentration (POC FLT) methods.
Results: Both POC-CCA had poor performances, showing low identification of less than half of positive individuals and several undiagnosed cases, revealing an accuracy of 0.44 and 0.46, and a Kappa Index of 0.308 and 0, respectively. Positivity rates of POC-CCA tests were below the one found for a single Kato-Katz slide. POC FLT had a Kappa Index of 0.617, an accuracy of 0.81, 67% of reproducibility, and was shown to have the same sensitivity of 21 Kato-Katz slides when two tests were performed.
Conclusions: POC-CCA® and POC Eco presented exactly the same inadequacy in low endemicity areas. POC FLT significantly improved the performance of POC-CCA®. More accurate methods must be evaluated in low endemicity areas.

1. Introduction

The World Health Organization identified schistosomiasis as the second most important human parasitic disease in the world, after malaria. Although the current prevalence is difficult to measure, 74 countries are endemic with approximately 207 million people affected in tropical countries, 120 million individuals being symptomatically infected and approximately 20 million being severely affected (Engels et al., 2002; Chitsulo et al., 2004; Steinmann et al., 2006). In some countries, elimination is already the declared goal (World Health Organization, 2017). Even in places where morbidity control is the main strategy, it is essential to ensure that adequate tools for transmission control and accurate identification of infected individuals are associated (Grenfell et al., 2012). Prevalence is expected to be low in several areas either under advanced control of transmission or with recent introduction. A high number of individuals that are particularly hard to diagnose by conventional methods are located in those areas, currently represented by the majority of the endemic fields in Brazil, Egypt and the People's Republic of China. Without proper diagnosis, they remain untreated completing the parasite life cycle.

Kato-Katz is the field method for detecting *Schistosoma mansoni* since 1980. It is a method of simple execution that identifies parasite eggs in stool samples (Katz et al., 1972). Although it is simple and low cost, it requires trained technicians in microscopy, and it may miss light infections. Consequently, Kato-Katz method based surveys and official programs that use one or two slides can underestimate local prevalence and leave individuals with low parasite burden undiagnosed (Grenfell et al., 2012; Siqueira et al., 2015; Enk et al., 2008).

POC-CCA® is a new method that has been tested in African endemic areas and most recently in Brazil. It is an easy-to-do

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https://doi.org/10.1016/j.jim.2019.06.006
Received 29 March 2019; Received in revised form 3 June 2019; Accepted 4 June 2019
Available online 10 June 2019

immunochromatography-based test that identifies the Circulating Cathodic Antigen, or CCA, in urine samples. Despite the satisfactory performance in moderate and high endemicity areas (Colley et al., 2013; Coulibaly et al., 2011, 2013; Casacuberta et al., 2016; Kittur et al., 2016; Lamberton et al., 2014; Foo et al., 2015; Shane et al., 2011; Adriko et al., 2014; Ochodo et al., 2015), it presents several problems in the low endemicity ones (Foo et al., 2015; Bezerra et al., 2018; Lindholz et al., 2018; Grenfell et al., 2018; Coelho et al., 2016). CCA testing has been shown to detect a significantly lower proportion of *S. mansoni* infections than Kato-Katz (1 or 2 slides) for low intensity infections. It also has no reproducibility for two or more cassettes and frequently shows different diagnosis when performed by different technicians (Grenfell et al., 2018; Coelho et al., 2016). Plus, a high number of faint trace results appear randomly on the analysis of negative and positive samples (Colley et al., 2013; Coulibaly et al., 2011, 2013; Casacuberta et al., 2016; Kittur et al., 2016; Lamberton et al., 2014; Foo et al., 2015; Shane et al., 2011; Adriko et al., 2014; Ochodo et al., 2015; Bezerra et al., 2018; Lindholz et al., 2018; Grenfell et al., 2018; Coelho et al., 2016), providing no guarantee on the decision of sending the individual to drug treatment.

The increasing number of individuals showing low parasite load, most of the time with less than ten eggs per gram of feces (identified after an extensive parasitological examination), turns the diagnosis even harder to be performed. Although it is considered the reference method for schistosomiasis, the Kato-Katz method requires a high number of slides to reach significant accuracy in areas of low endemicity (Siqueira et al., 2015; Enk et al., 2008; Lindholz et al., 2018; Grenfell et al., 2018; Siqueira et al., 2016). POC-CCA®, on the other hand, presents a persistent low accuracy even after two or more cassettes are used (Grenfell et al., 2018; Coelho et al., 2016). Therefore, we hereby report the results of the application of POC-CCA®, the Urine CCA (Schisto) ECO Teste®, a new commercially point of care option for CCA detection produced in Brazil and licensed in 2017, and the POC FLT that uses the same POC-CCA® cassette with a 10×-concentrated by filtration urine for individuals with low parasite load (Grenfell et al., 2018). Both methods were compared to the Kato-Katz and Saline Gradient parasitological methods.

2. Materials and methods

2.1. Study locations and sample collection

Two areas located in the rural area of Minas Gerais, in a distance of approximately 500 km from the state capital Belo Horizonte in the southeast of Brazil were selected to participate in this prospective survey. The population presented a low migration index, and the survey was conducted in 2015–2016. As previously described (Grenfell et al., 2018), both the selected areas had different profiles.

Estreito de Miralta, a village with 163 residents, showed a schistosomiasis prevalence rate of 10.34% in 2008 and of 30% in 2016 after an intensive search for eggs. Residents had not received treatment for schistosomiasis in the previous two years. Infected patients were treated with praziquantel (single oral dose – 60 mg/kg for children and 50 mg/kg for adults). The total amount of residents participating was 84 individuals (46 females and 38 males, 1–86 years old).

Samambaia is a village close to Estreito de Miralta with 179 inhabitants (79 females and 100 males, 2–84 years old). It is a peculiar area with no helminths, despite the location and appropriate environment.

Urine and feces samples from 48 residents of this two areas were selected for this study, including 27 schistosome positive and 21 schistosome negative individuals (21 female, 27 male, 4–81 years old). Urine samples were collected on the same day of the feces collection in 2016, conserved in a freezer (−20 °C) in separate aliquots and immediately used after defrosting at room temperature.

2.2. Parasitological methods

Positivity was defined as any positive parasitological slide performed for each stool sample by Kato-Katz (Katz et al., 1972) and Saline Gradient (Coelho et al., 2009) techniques. Each resident provided feces from which 24 slides of Kato-Katz thick smear were prepared for examination (totalizing 1 g of feces, 24 × 42 mg) (Helm-Test®, Biomanguinhos, FIOCRUZ, Brazil). For both methods, results were expressed as eggs per gram (epg) of feces.

Kato-Katz was performed according to the manufacturer's instructions (Katz et al., 1972). Briefly, each sample was placed on a paper, then a metal mesh was pressed over it. Feces were then scraped into a plastic circular template mounted on a glass slide so that the template was filled. After removing the template, cellophane coverslips presoaked in a glycerin-malachite green solution were placed over the sieved feces. Each slide was completely screened by optical microscopy at a final magnification of 100× for the identification and quantitation of eggs.

Each sample was also used in parallel for two analysis of 500 mg each by Saline Gradient technique (Coelho et al., 2009). Briefly, device was set up with a 3% saline solution separating column. The excess saline was discarded in the separation area using a pipette. Then, the separating column was filled with the fecal suspension previously prepared by a dilution of a 500 mg stool sample in 3 mL of 0.9% saline solution. Finally, the roller clamp was opened for adjustment of the saline flow to 10 drops/min. The slow and continuous flow of the 3% saline solution from the reservoir column to the separating column cleaned the sediment in the bottom of the latter column. Schistosome eggs that have a higher density remained over the surface of the porous plate and the final sediment was further transferred to a glass slide and examined under a bright field microscope for the presence of eggs.

2.3. POC-CCA®

Urine samples were used to compare all three POC tests in duplicate analysis by three different readers in blind conditions. One reader was a pharmacist, one was a laboratory technician and the last one was a technician from ECO Diagnostica. POC-CCA® tests were performed according to the manufacturer's instructions (Rapid Medical Diagnostics, lot #50173, Pretoria, South Africa). Briefly, one drop of urine was added to the testing well on the cassette. After absorption, a drop of the kit buffer was added into the well. Results were read after exactly 20 min. Results were scored as negative if the result revealed no test line, as trace if a very light test line appeared and as positive if a clear test line appeared.

2.4. Urine CCA (Schisto) ECO Teste®

Urine CCA (Schisto) ECO Teste® is manufactured in Brazil by licensed of Rapid Medical Diagnostics, titled here as POC Eco, was also performed according to the manufacturer's instructions (ECO Diagnostica, lot#170522062, Corinto, Brazil). Three drops of urine was added to the well on the cassette. No buffer was used. Results were read in 20 min. Results were scored as negative for a color test line, as trace if a very light test line appeared and as positive if a clear test line appeared.

2.5. POC FLT

POC FLT was done as described previously (Grenfell et al., 2018). Briefly, urine was ten times concentrated in a 30 kDa filter (MRCF0R030, Merck Millipore, Germany) from 500 µl by centrifugation in a bench microcentrifuge (Gilson PMC880) for 30 min. The pellet was resuspended in 50 µl of distilled water, and a drop of the concentrated sample was applied on the POC-CCA® cassette. After absorption, a drop of the kit buffer was added into the well. Results were

read after exactly 20 min. Particularly for POC FLT, traces are defined as negatives. So results were scored as negatives for no colored test line or a faint test line and, as positive for a clearly colored test line.

2.6. Data analyses

POC tests were analyzed in comparison to the parasitological ones. The reference was defined as any positive slide performed for each feces sample by Kato-Katz and/or Saline Gradient techniques. Software Prism 5.0 was used to determine accuracy and to perform other statistical analysis. Comparison between methods was done by One-Way ANOVA using Tukey's multiple comparison correction. The agreement between the parasitological methods and POC tests were assessed by Kappa Index calculated by GraphPad (GraphPad Software, Inc., USA): $k < 0.01$ no agreement; $k = 0.01–0.20$ 'poor'; $k = 0.20–0.40$ 'fair'; $k = 0.40–0.60$ 'moderate'; $k = 0.60–0.80$ 'substantial'; $k = 0.80–1.00$ 'almost perfect'(Landis and Koch, 1977).

3. Results

A total of 48 urine samples from Estreito de Miralta and Samambaia residents previously diagnosed were selected for this analysis. Kato-Katz and Saline Gradient methods were used together as a reference to determine positivity rates in 1 g of feces samples for each method. Parasitological methods detected 21 positive individuals ranging from 1 to 76 *S. mansoni* epg and 27 negative individuals. Hookworms were detected in three stool samples (2 *S. mansoni* positives and 1 negative) and *Enterobius vermicularis* in 2 *S. mansoni* positive samples. Table 1 presents the raw data from the diagnosis of the 48 individuals by parasitological analysis and each of the three POC tests performed in 2016. As shown, diagnoses obtained from the three POC tests were diverse. POC-CCA® presented a high number of doubly traces 18/48 representing 37.5% of samples and only 10/21 positive individuals. POC Eco presented an elevated number of negatives (33/27), undoubtedly presenting false-negative results. POC FLT, on the other hand, presented similar diagnosis to the reference with 28/27 negative and 20/21 positive individuals.

Accuracy and Kappa Index predictors were used to compare diagnosis results. POC-CCA®, POC Eco and POC FLT had accuracy of 0.44, 0.46 and 0.81, respectively (Table 2). Together, the Kappa Index analysis showed 'fair' agreement for POC-CCA® ($k = 0.31$, 95% confidence interval), 'moderate' agreement for POC FLT ($k = 0.62$) and 'poor' agreement for POC Eco ($k = 0$).

Reproducibility was measured by comparing the diagnosis performed by the three different readers/technicians (Table 3). POC-CCA®, POC Eco and POC FLT presented 18/48, 34/48 and 32/48 unanimous agreements, against 30/48, 14/48 and 16/48 disagreements between two readers. For POC-CCA®, 27% were trace/negative and 73% trace/positive divergent readings. POC Eco presented 79% of trace/positive and 7.5% of both trace/negative and negative/positive, while POC FLT showed only trace/positive divergences. No disagreement was notified among the three readings for the same POC test. Otherwise, a high number of 13 readings was in complete disagreement when comparing the mode of the readings between the three POC tests, showing positive, negative and trace results for the same samples. Among those, POC FLT gave the right diagnosis for 12 individuals (four negatives and eight positives, with 1–76 epg). POC-CCA® correctly diagnosed 2 negative individuals and, POC Eco 3 individuals (2 negatives and 1 positive with 9 epg).

When we divided the 21 positive individuals by the epg, we found important differences in each POC test sensitivity (Table 4). POC FLT presented a significantly higher capability of diagnosing a very low schistosome burden with the detection of 75% of positive individuals (epg < 10) than other two POC tests (25% and 12.5%, for POC-CCA® and POC Eco). Same significant differences were seen for epg > 10 with the identification of 80% of the individuals in comparison to the 20% detected by POC-CCA® and POC Eco. POC-CCA® presented the highest number of traces (56 and 60%, for epg < 10 and > 10, respectively) in comparison to POC Eco (12.5 and 40%). POC Eco, instead, presented the highest number of false negatives, with 75% of the individuals with epg < 10.

The estimates of egg positive individuals with low parasite burden were determined by the number of cassettes performed for each sampleand can be seen in Table 5.

The use of two POC-CCA® cassettes increased not significally the identification to 29%. Interestingly, POC FLT presented an important capability for diagnosing low schistosome burden ranging from 88% for one single test, being equivalent to 18 Kato-Katz slides, to 94% when a second test was done having the same positivity rate of 21 Kato-Katz slides. ECO Diagnostica did not provide extra cassettes for POC Eco examination and had a poor performance with only 14% of positivity for a single cassette. Kato-Katz, as the reference method, needed the examination of 24 slides to identify all the positive patients (Table 6).

Table 1
Diagnosis for *Schistosoma mansoni* using Kato-Katz and Saline Gradient for feces samples, POC-CCA®, POC Eco and POC FLT for urine samples of Estreito de Miralta and Samambaia individuals, Minas Gerais, Brazil.

	Kato-Katz (24 slides, 1 g of feces) + Saline Gradient (1 g of feces)	POC-CCA®	POC Eco	POC FLT
Negative	27	20	33	28
Positive	21	10	9	20
Trace	–	18	6	0[a]
Total	48			

[a] Traces after urine concentration by POC FLT are negative.

Table 2
Accuracy of POC-CCA®, POC Eco and POC FLT evaluated against a combined gold standard of 24 Kato-Katz slides and 2 Saline Gradient analysis.

	Accuracy	Kappa index
POC-CCA®	0.44	0.31 Fair
POC Eco	0.46	0.00 No Aggreement
POC FLT	0.81	0.62 Moderate

Table 3
Reproducibility of POC-CCA® and POC FLT for 32 individuals from Estreito de Miralta and Samambaia/MG evaluated at two timepoints (March/2017 and March/2018).

	POC-CCA®	POC FLT
Reproduction	12	25
Disagreement	20	7
Negative/positive	2	1
Trace/Negative	10	–
Trace/Positive	8	6

4. Discussion

Rapid accurate POC tests for schistosomiasis would be valuable for field diagnosis. However, to achieve the whole number of positive individuals in many countries, particularly for low endemicity sets, higher sensitivity and no doubly trace readings will likely be required. This strategy will enable diagnosis and individualized treatment, potentially interrupting the parasite life cycle, reducing morbidity and consequently improving the quality of life of endemic residents.

Here, we characterized important properties of two rapid tests commercially available and one innovative optimized option. The study design had three overall aims: 1) to evidence the efficiency of each POC test based on a strong reference criteria that included two

Table 4
Distribution of POC-CCA®, POC Eco and POC FLT results according to egg burden (epg ≤10 and > 10) in 21 Kato-Katz and/or Saline Gradient positive samples collected from Estreito de Miralta, Minas Gerais.

	POC-CCA®				POC Eco				POC FLT			
Detection result	epg ≤ 10	%	epg > 10	%	epg ≤ 10	%	epg > 10	%	epg ≤ 10	%	epg > 10	%
Positive	4[a]	25	1	20	2	12.5	1	20	12[b,c]	75	4[b,c]	80
Negative	3	19	1	20	12[a,c]	75	2	40	4	25	1	20
Trace	9[a]	56	3	60	2	12.5	2	40	–	–	–	–
Total	16	100	5	100	16	100	5	100	16	100	5	100

epg, egg per gram of feces. a, b and c indicate significant differences respectively for POC-CCA x POC Eco, POC-CCA x POC FLT and POC Eco x POC FLT ($p < 0.03$), One-Way ANOVA.

Table 5
Percentage of positive individuals detected by 1 or 2 cassettes of POC-CCA®, POC Eco and POC FLT.

	1 cassette	2 cassettes
POC-CCA®	24%	29%
POC Eco	14%	ND
POC FLT	88%	94%

ND, Not done.

Table 6
Percentage of the 21 positive individuals detected by increasing number of Kato-Katz slides.

1 slide	2 slides	10 slides	18 slides	21 slides	24 slides
50%	63%	75%	88%	94%	100%

parasitological methods extensively repeated for each individual (totalizing 2 g of examined feces), 2) to identify reproducibility, and 3) to identify which POC test present a trustable performance for low burden individuals and are suitable for field application in countries of low endemicity. The data demonstrated that the choice of the method is critical for the achievement of a sensitive and reliable schistosomiasis diagnosis, since commercially available POC tests present inconsistent results.

POC-CCA® is a rapid antigen-based detection test, produced in South Africa, that uses urine samples to promote the detection of a schistosome antigen. It has received increasing attention as a promising point of care field diagnostic tool (Lindholz et al., 2018). Though it has been presenting good sensitivity in areas ranging from moderate to high endemicity (Colley et al., 2013; Coulibaly et al., 2011, 2013; Casacuberta et al., 2016; Kittur et al., 2016; Lamberton et al., 2014; Foo et al., 2015; Shane et al., 2011; Adriko et al., 2014; Ochodo et al., 2015), evaluations of this test in areas of low endemicity have begun to demonstrate its lack of efficiency (Bezerra et al., 2018; Lindholz et al., 2018; Grenfell et al., 2018; Coelho et al., 2016; Siqueira et al., 2016). Brazil is endemic for schistosomiasis, with 14 endemic states that cover rural and urban areas of low endemicity. As shown by the National Prevalence Survey of Schistosomiasis and soil transmitted helminths (Katz, n.d.), the national positivity rate for schistosomiasis is currently 0.99%. This positivity rate decreased from 10.09% (1949–53) to 9.24% (1975–78) and finally to 1.79% (2010–15) in the 11 main schistosomiasis endemic Brazilian states. Therefore, it is vital to evaluate all available POC options in populations that hold profiles different from the already studied African sets and to consider data obtained from different emerging new surveys (Bezerra et al., 2018; Lindholz et al., 2018; Grenfell et al., 2018; Coelho et al., 2016; Siqueira et al., 2016).

In this current study, raw data analysis indicated that POC-CCA® presented almost 40% of undefined diagnosis and was able to identify < 50% of true positive individuals for patients with 1–76 epg. Due to the low amount of circulating antigens on urine of individuals with low parasite burden, both POC tests failed on having a high sensitivity. Individual analysis indicated accuracy lower than 0.50 with a 'fair' agreement with the associated parasitological methods used here as a reference. This lack of correlation was seen before (Bezerra et al., 2018; Lindholz et al., 2018; Grenfell et al., 2018; Coelho et al., 2016; Siqueira et al., 2016). Some of these studies have shown the identification of 4% and 10% of positive individuals (Bezerra et al., 2018; Lindholz et al., 2018), respectively, and a poor consistency between Kato-Katz and POC-CCA® (Bezerra et al., 2018; Lindholz et al., 2018; Grenfell et al., 2018; Coelho et al., 2016). Lindholz et al. (2018) showed that the performance of the POC-CCA® method was worse in the subset of samples that contained < 1 epg, with a lower detection rate of true positives and a higher number of false positives (Lindholz et al., 2018). We found a similar inadequacy when the epg is lower than 10, with 25% of true positives correctly identified, 19% non-identified and 56% with undefined diagnosis. The same was noticed for individuals with 11–76 epg with 20% positives, 20% non-identified and 60% undefined diagnosis. Finally, increasing the number of cassettes does not make a significant difference on POC-CCA® effectiveness as the percentage of positive detected individuals changes from 24% to 29% for a two cassettes diagnosis, being below the detection rate of the 50% detected by a single slide of Kato-Katz. The same evaluations were done for POC Eco, which is also a rapid test produced in Brazil that has the same purpose of detecting the CCA in urine. The difference here is that this test uses three drops of urine and no buffer. As an emerging new test, it has not been evaluated before in any other study. According to the manufacturer, it has a higher sensitivity than POC-CCA® and Kato-Katz (1–2 slides). Our results are in disagreement with this statement as we found 22% of false negative and 12.5% of false positive individuals. Plus, accuracy was 0.46, similar to the 0.44 of the POC-CCA®, and the Kappa Index was zero (k = 0) showing no agreement with the Kato-Katz reference test. Kappa Index analysis showed that POC-CCA® had better agreement with Kato-Katz than POC Eco. Although recently a version of the POC Eco provided by the manufacturer was supposedly developed to be cleared of the "trace" readings, of all positive individuals, only 12.5% and 20% were correctly diagnosed by the POC Eco, respectively, for individuals with epg < 10 and epg > 10. Several positive individuals with < 10 epg were not diagnosed presenting 75% of them as negatives and 12.5% as undiagnosed. Similarly, 40% of individuals with 11–76 epg stayed as negatives and other 40% as undiagnosed. Finally, when only one cassette of POC Eco was used, a poor 14% of positivity was found, being significantly below the 50% of a single Kato-Katz slide.

POC-CCA® failed on the reproducibility test on 63% of the cases, including egg negative and positive individuals, when disagreements among readers were trace/negative and trace/positive. POC Eco presented the lowest number of disagreements (14/48), but with its poor performance related here, agreements on erroneous diagnosis stand as irrelevant.

POC FLT is an optimized methodology adopted by our group to improve POC-CCA® sensitivity that uses a urine concentration step. In a previous work, POC FLT was evaluated with 183 samples from two

Brazilian endemic areas with different profiles, and it was demonstrated to have high accuracy and trustable positivity rates (Grenfell et al., 2018). Here, we had the opportunity to compare its performance to the two available POC tests. POC FLT provided similar diagnosis than the rigorous reference methods adopted here (24 Kato-Katz slides + two Saline Gradient analysis), identifying 28/27 negative individuals and 20/21 positive individuals with an accuracy of 0.81. Also, 67% of unanimous reproduction among readers were obtained. Grenfell et al. (2018) showed an accuracy of 0.86 and 0.96 of POC FLT on two distinct Brazilian endemic areas against 0.51 and 0.36 of POC-CCA®. Similarly, Lindholz et al. (2018) showed that accuracy ought to be increased when they found 0.56–0.64 for POC-CCA® in another Brazilian endemic set in Sergipe state that ought to be increased (Lindholz et al., 2018). When exclusively comparing positivity rates obtained by one cassette of POC FLT to Kato-Katz, 88% was significantly higher than the 50% from only one Kato-Katz slide. This positivity rate of 88% from one POC FLT was the same obtained by 18 Kato-Katz slides. When two POC FLT analysis were done, this rate was increased to 94% being equivalent to 21 Kato-Katz slides. As no significant difference was noticed for these rates of 88% and 94%, one can assume that only one cassette of POC FLT is enough for a survey with no requirement of doubling the kits used, so controlling the costs. Although POC FLT increases the time of conventional immunochromatography by 30 min, it increases sensitivity for diagnosing hard-to-detect patients (with parasite burdens extremely low as −1–10 epg) and is two times more sensitive than the WHO preconized method (Kato-Katz, one slide) (World Health Organization, 2017, 2018). These conclusions are evidenced by the identification of 75% of individuals with epg < 10 (against 25% of POC-CCA® and 12.5% of POC Eco) and 80% of individuals with 11 > epg < 76 (against 20% of both POC tests). Bezerra et al. (2018) found 10/258 positive individuals with an arithmetic mean of 8 epg by using POC-CCA® and, although the identification of positives was superior to Kato-Katz (4/258), only three slides of feces were analyzed, leading to inconclusive results, as for six individuals it is not possible to know if they were truly positive (low burden) or negative (Bezerra et al., 2018). Studies performed in different African settings (Colley et al., 2013; Coulibaly et al., 2011) where *S. mansoni* is endemic have repeatedly demonstrated that the POC-CCA® is capable of detecting additional positive cases when compared to two or three Kato-Katz thick smears. As shown (Enk et al., 2008; Grenfell et al., 2018; Coelho et al., 2016; Siqueira et al., 2016), there is an important advantage of an increased Kato-Katz sampling effort, as means of control programmes or as a reference to evaluate additional new methods. This is especially applied for low endemic areas, where the cost-effectiveness of the strategy of using no more than one or two parasitological slides becomes questionable.

5. Conclusions

Priority needs to be given to affordable POC solutions that can be used in a variety of settings, in high, low and middle income countries as part of the schistosomiasis control programmes. Elimination is already the declared goal in Brazil, as recommended worldwide by WHO. In order to achieve this, an urgent improvement of the diagnosis methodologies has to be accomplished (Silva-Moraes et al., 2014). The Kato-Katz technique remains the gold standard for the detection of schistosome eggs in stool samples. However, due to the small amount of feces per slide and the low egg production by individuals in low endemic areas, the risk of having a large number of individuals who remain undiagnosed is considerable. Equally, two available POC tests demonstrated significant low sensitivity and accuracy, no trustful reproducibility, and a relevant number of double results, leading to undiagnosed and untreated individuals. One cannot agree on having new methods with similar deficient performance introduced as substitution of the conventionals in control programmes of countries where endemic sets are distinctly different from the so far tested without proving the benefits. Recommendation is to firstly have innovative POC alternatives not only providing rapid diagnoses, which is critical for improving general public health in resource-limited settings, but also providing efficiency on the diagnosis of any positive individual and for the control of cure. New alternatives must be evaluated as the new *Schistosoma* up-converting phosphor lateral flow circulating anodic antigen (UCP-LF CAA) assay (van Grootveld et al., 2018; Corstjens et al., 2017) and improved POC methodologies. Costs should be evaluated as well. By showing POC FLT results, it was demonstrated that POC-CCA® has the potential to be used, but not as it is now. It needs to be improved for a higher sensitivity. In the meantime, it is required that low endemicity countries improve schistosomiasis diagnoses when individuals with low parasite burdens are in evidence, by using an increased number of Kato-Katz thick smears or associating different tools as Helmintex (Lindholz et al., 2018), Saline Gradient (Coelho et al., 2009) methods and others, although we recognize that this implies on a great effort by developing countries.

Authors' contributions

Conceptualization: RG, NK, PMC
Data curation: RG, FC, MLP, AA, NK, PMC
Formal analysis: RG, NK, PMC
Funding acquisition: RG, NK, PMC
Investigation: RG, FC, MLP, AA, NK, PMC
Methodology: RG, FC, MLP, AA
Project administration: RG, PMC
Resources: RG, NK, PMC
Supervision: RG, FC, NK, PMC
Validation: RG, FC, MLP, AA, NK, PMC
Visualization: RG, FC, MLP, AA, NK, PMC
Writing - original draft: RG
Writing - review & editing: RG, NK, PMC

Funding

The Brazilian National Council for Scientific and Technological Development (CNPq), Minas Gerais Research Foundation (Fapemig), Oswaldo Cruz Foundation (FIOCRUZ).

Declaration of Competing Interests

The authors have declared that no competing interests exist.

Ethical approval

This work has the approval of the Research Ethics Committee of the Rene Rachou Research Center (CEPSH/CPqRR 03/2008). Study objectives were explained to the participants in the endemic field who signed the written informed consent form before being admitted to the study. Parents/guardians provided written consent on behalf of all participants under 18 years of age. Children received an explanation about the procedure in a clearly explained language and had the right to express their opinion after parents/guardians had signed the informed consent. All the procedures were performed in the presence of parents/guardians. Fecal and urine samples were coded by numbers and the results treated confidentially. All infected participants (by *S. mansoni* and other helminths defined by the parasitological methods) were treated as recommended by the Brazilian Health Ministry, as previously described (Grenfell et al., 2018).

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