Diagnosis and post-treatment monitoring of schistosomiasis in endemic and non-endemic settings by quantification of schistosome circulating anodic antigen

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Part III.

Reflections on circulating anodic antigen detection for context-specific diagnosis of schistosomiasis

8.

Summarizing discussion

Adapted and extended from:
Context-Specific Procedures for the Diagnosis of Human Schistosomiasis – A Mini Review

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Frontiers in Tropical Diseases (2021)

The use of sensitive and specific diagnostic tests for schistosomiasis, to correctly identify those who are infected, is crucial to successfully reduce the burden of disease and to eventually move towards elimination of schistosomiasis. However, the diagnostic need differs between settings with each setting requiring its own distinctive approach. In addition to accurately diagnosing schistosomiasis, monitoring the efficacy of praziquantel (PZQ) treatment also requires highly accurate diagnostic methods. It is generally acknowledged that traditional microscopy lacks sensitivity in detecting low intensity infections, as is often the case after treatment. Since PZQ is known to target adult schistosome worms, accurately measuring the worm burden would be a more direct way to determine efficacy of treatment, compared to determining number of eggs (often used as a proxy for worm burden). In this thesis, we evaluated the performance of the UCP-LF CAA test in the context of endemic and non-endemic settings for diagnosing schistosomiasis and monitoring efficacy of PZQ treatment. The UCP-LF CAA test is a lateral flow (LF) test for quantitative detection – using luminescent high sensitivity up-converting reporter particles (UCP) – of circulating anodic antigen (CAA) regurgitated by live schistosome worms in the human circulation. As CAA is assumed to be excreted by all schistosomes, the UCP-LF CAA test is expected to be suitable for detection of infections with all *Schistosoma* species, including veterinary ones and hybrid infections (chapter 2). The presence of CAA, measured in urine and/or serum, reflects an active *Schistosoma* infection: CAA-levels decrease rapidly to low or undetectable levels after treatment thereby confirming the efficacy of PZQ (chapter 2, 3 & 6). In non-endemic settings (i.e. the absence of reinfection), undetectable CAA-levels indicate clearance of infection (chapter 2 and 3). This in contrast to endemic settings where – with continuous exposure and ongoing transmission – CAA-levels significantly decrease but often remain detectable (chapter 6). Similarly to egg microscopy, but with relevantly higher sensitivity, the UCP-LF CAA test not only indicates active infection but also allows better perception on the intensity (worm burden) of *Schistosoma* infections (chapter 7).

Using a composite reference standard in diagnostic evaluation studies

In all studies, the UCP-LF CAA test showed a significantly higher number of positive cases compared to other diagnostic methods, regardless of the setting. The most important indication for the UCP-LF CAA test being more accurate is the fact that CAA-levels significantly decrease after PZQ treatment, confirming that CAA is excreted by live worms and indicating the effect of treatment on active *Schistosoma* infections (chapter 2, 3 & 6). Still, it remains important to have an accurate estimation of the sensitivity and specificity of the UCP-LF CAA test. In order to obtain this, the UCP-LF CAA test was compared against a composite reference standard (CRS) in three different studies described in this thesis (chapter 3, 6 & 7). This is a commonly applied approach when there is no single suitable reference standard available [1,2] and has already been used in several schistosomiasis diagnostic evaluation studies [3-5]. It is an alternative to, for example, latent class analysis, since the conditions for this kind of statistical analyses are often difficult to meet in particular in studies evaluating diagnostic methods for schistosomiasis [6]. For example, there is conditional dependence among diagnostic tests, the sample size is often too small, and the number of different tests available for the analysis is insufficient.

In all studies (chapter 3, 6 & 7), the CRS consisted of a set of diagnostic tests that were assumed to have or approach a specificity of 100%. Depending on the type of diagnostic tests included in the CRS, an individual was classified as true positive based on the OR rule (i.e. individuals with at least one positive test were classified as disease positive and those with all negative tests were classified as disease negative, chapter 6 & 7) or the K rule (i.e. disease positive if at least K tests were positive, chapter 3). Even though the UCP-LF CAA test was being investigated, it was decided to include the test in the CRS based on the fact that in previous studies the detection of CAA has proven to be highly specific [3-5,7-11], likely due to the chemically unique structure of CAA which has not been observed in organisms other than schistosomes [12]. Nevertheless, it is important to keep in mind that this could potentially have led to an overestimation of the sensitivity of the UCP-LF CAA test. It was difficult to compare the performance of the UCP-LF CAA test between different studies as the reported accuracy of the test also depends on the disease prevalence which varied between the studies described in this thesis. However, despite these differences in prevalence and setting, the sensitivity of the UCP-LF CAA in the studies described in this thesis was consistently high (>90%), similar to previous findings [3,8,13,14].

Performance of circulating anodic antigen detection in non-endemic settings

Distinctive populations can be identified within non-endemic settings, primarily short-term travelers (including tourists and expatriates) and migrants (including refugees). In general, travelers have not been exposed previously and are therefore considered to be immune-naïve, whereas migrants, when originating from *Schistosoma* endemic areas, have often been exposed since childhood and are more likely to present with chronic infections. In exceptional cases, migrants originating from non-endemic schistosomiasis areas may have acquired an acute *Schistosoma* infection when passing through a schistosomiasis endemic area. In areas not endemic for schistosomiasis, diagnosis is usually focused on test-and-treat of the individual patient, i.e. early detection of infection and confirmation of cure following intervention. The general aim within clinical settings is complete eradication of infection in each individual patient as there is no risk of reinfection.

Travelers

Only 30-50% of schistosome infected travelers present with clinical symptoms [15,16], but if they develop acute schistosomiasis, the so-called Katayama syndrome, it is generally seen several weeks before eggs can be detected. In combination with the low worm burden that travelers often harbor, this makes diagnosis with conventional microscopy challenging [9,16-18]. Since seroconversion usually occurs within 4 to 8 weeks after exposure, detection of schistosome-specific antibodies is the most commonly used alternative diagnostic approach for diagnosing schistosomiasis in previously naïve individuals. However, a major disadvantage of antibody detection methods is that they cannot distinguish between current and past infection nor provide any information regarding the intensity of infection. Alternatively, detection of *Schistosoma* DNA in blood has shown to be highly sensitive and specific for early diagnosis of acute schistosomiasis, but also this PCR outcome remains positive for many months after treatment [19,20]. In chapter 2 the diagnostic value of CAA detection was evaluated against a panel of

antibody and DNA detection methods for early diagnosis of schistosomiasis and monitoring PZQ treatment efficacy in a cluster of exposed Belgian travelers with a confirmed hybrid infection between *S. mattheei* x *S. haematobium* infection [20]. The UCP-LF CAA test in serum was the most sensitive test to confirm active *Schistosoma* infection as well as to assess cure: CAA-levels were detectable 4 weeks after exposure and decreased to undetectable levels following PZQ treatment, as also observed in previous studies [9,10,21]. Even though in this study it appeared to be a hybrid infection, CAA was detected in serum of all infected individuals, confirming that these *Schistosoma* species also excrete CAA [22-24]. The UCP-LF CAA test is not only suitable for detecting active infection in (recently) exposed travelers, but interestingly it can also be used to demonstrate the absence of an active infection in travelers who for example show a positive serology, but have no additional indications of harboring viable worms.

Migrants

The increasing number of migrants, coming from or passing through *Schistosoma* endemic regions and arriving in Europe, stresses the importance of timely and effective screening for *Schistosoma* infections [25-27]. Detecting *Schistosoma*-specific antibodies remains the recommended and most used first-line test for screening migrants [28,29]. Despite being the most commonly used diagnostic method for imported infections, antibody detection procedures still have their limitations both in sensitivity and specificity for suspected infections. It is common practice in many settings to use the detection of eggs in urine or stool as a confirmation test to prove an active infection, despite the fact that it is generally acknowledged that microscopy lacks sensitivity [30]. A recent study in migrants showed that the point-of-care circulating cathodic antigen (POC-CCA) test – a commercially available and field-applicable rapid test particularly suitable for diagnosing intestinal schistosomiasis – can, in combination with serology, be an efficient screening tool for *S. mansoni* infections when used in a standardized manner [31-33]. Additionally, the detection of *Schistosoma* DNA by the *Schistosoma* genus-specific ITS2 real-time PCR in stool and urine sample has demonstrated to be of clinical value when monitoring schistosomiasis in migrants after their arrival in Europe [34]. In contrast to the real-time PCR in blood which – despite being highly sensitive and specific for diagnosing schistosomiasis – remains positive after treatment [19,20], there are strong indications that the clearance of *Schistosoma* DNA in stool or urine occurs within weeks to months following PZQ treatment [35]. This was confirmed in chapter 3 where a panel of diagnostics, including real-time PCR and circulating antigen (POC-CCA and UCP-LF CAA) diagnostics, were applied to samples from asymptomatic migrants. The majority of stool PCR-positive individuals became negative after treatment. However, the UCP-LF CAA test on serum seems more suitable for migrants originating from different schistosomiasis endemic regions as CAA is excreted by all *Schistosoma* species including hybrids (shown in chapter 2), is detectable even in low intensity infections [10,34] and simply because serum is the preferred sample type from a routine diagnostic procedure perspective. In chapter 3 the UCP-LF CAA test was further investigated and confirmed the previously observed high microscopy-based prevalence of *S. mansoni* infections in asymptomatic migrants. CAA results showed a good agreement with microscopy as well as with serology, real-time PCR and POC-CCA results. However, almost half of the CAA-positive cases could not be confirmed with microscopy nor real-time PCR, and even

though a slightly better overlap was observed with POC-CCA results, this still implied that a significant amount of *Schistosoma* infections had been missed. Also here a significant decline to very low or undetectable CAA-levels was observed after treatment, indicating that an active infection was present which was cured after treatment, similar to previous findings [9,34,36]. Compared to diagnostic methods currently used in routine diagnostic settings (in particular antibody detection and microscopy), the UCP-LF CAA test is more suitable for diagnosing *Schistosoma* infections as well as to determine individual cure shortly after treatment. Especially in the migrant population, a short follow-up period is preferable. Newly arrived migrants tend to be very mobile during the first months to years after their arrival, so this population would benefit most from a diagnostic test that is able to determine efficacy of treatment (i.e. clearance of infection) within weeks, allowing additional treatment if needed.

Performance of circulating anodic antigen detection in endemic settings

In schistosomiasis endemic regions more attention is generally given to diagnosis for public health purposes than to the identification of individual cases. In such settings, determination of an infected individual is often based on clinical symptoms only, sometimes combined with the detection of eggs in stool or urine. From a public health perspective, the absence or a significant reduction in the number of *Schistosoma* eggs is crucial as eggs are the cause of morbidity and ongoing transmission. The cornerstone of schistosomiasis control in endemic settings mainly relies on large-scale administration of PZQ at regular intervals to at-risk populations aiming to decrease and keep the overall worm burden low within the population [37-40]. The efficacy of mass drug administration (MDA) with PZQ is commonly evaluated by detection of parasite eggs in urine or stool [41-43]. Due to the reduced sensitivity of microscopy, in particular in case of low infection intensity, previously reported efficacy of MDA has been overestimated [44-47]. Repeating treatment at short intervals in regions with ongoing transmission could potentially increase the efficacy of PZQ, as non-susceptible schistosomula [48] – who within a few weeks will have matured into drug susceptible worms – are targeted as well. This approach of repeating PZQ treatment was investigated in a group of school-aged children with a confirmed *S. mansoni* infection. To this end, an open-label, randomized controlled clinical trial called 'Repeated doses of Praziquantel in Schistosomiasis Treatment' (RePST) was conducted in Côte d'Ivoire (chapter 4). Over the course of a 10-week period, children received either a single PZQ treatment or four repeated PZQ treatments at 2-week intervals. A wide range of diagnostic methods was applied in this study, classified as egg-based diagnostics (i.e. Kato-Katz and real-time PCR) and worm-based diagnostics (i.e. circulating antigen detection methods: POC-CCA and UCP-LF CAA). Chapter 5 subsequently describes the initial results from the diagnostic methods used in the field, i.e. Kato-Katz and POC-CCA. After a single treatment, the cure rate as well as the intensity reduction rate based on Kato-Katz were found to be similar compared to previous findings [41]. However, the majority of children remained positive by POC-CCA even after repeated treatment, resulting in very poor cure rates. Therefore, more accurate methods (i.e. real-time PCR and UCP-LF CAA) were applied to investigate to what extent POC-CCA positives reflected true infections (chapter 6). Urine CAA-levels correlated well with egg counts in feces, in particular in increasing infection intensity, confirming previous observations [49-55]. Nonetheless, discrepancies between the different diagnostic methods occurred, a phenomenon

that is inherent to diagnostic evaluation studies. Of particular interest were egg-positive cases with no detectable CAA in urine or serum. This disagreement of the absence of CAA while eggs have been observed could be due to passing of remaining eggs while the worms already died or variation in biological excretion of CAA [56,57], but administrative errors such as sample processing and or labeling cannot be excluded either. In contrast, the opposite – CAA-positive but no eggs in stool or urine – was observed more often. Since CAA is a worm-derived antigen, its presence is indicative of an active *Schistosoma* infection. Nevertheless, it is important to note that different schistosome life stages are being measured, namely the detection of eggs in stool or urine by microscopy versus the detection of worm-derived CAA in serum or urine by the UCP-LF CAA test. The absence of eggs while CAA is being detected can largely be attributed to the low sensitivity of microscopy, but could for example also be due to individuals harboring living (single sex) worms with only sporadically excreting eggs or with no detectable eggs at all [47,58-60] or a reduced fecundity due to PZQ treatment [61]. Overall, in chapter 6 we showed that PZQ efficacy measurements vary based on the diagnostic method that is used: while the cure rate determined by egg-based diagnostics (Kato-Katz, real-time PCR) significantly increased after repeated treatment, the cure rate determined by worm-based diagnostics (POC-CCA, UCP-LF CAA) remained poor over time. Even though all four diagnostic methods demonstrated a significant reduction in infection intensity already after a single treatment, the circulating worm-derived antigen diagnostics indicated the presence of active *Schistosoma* infections despite multiple treatments. CAA results confirmed the number of infected children to be abundant, even after repeated treatment, albeit with relatively low CAA-levels. Another recent study investigating the dynamics of parasite clearance and re-infection by Kato-Katz and POC-CCA also concluded that timing of post-treatment sampling is important as well as the diagnostic test used to determine cure rate and re-infection [62,63]. To better understand and optimize treatment strategies, highly sensitive methods such as real-time PCR and UCP-LF CAA should be used in conjunction to provide adequate insight into the host-parasite interaction and post-treatment dynamics of schistosome circulating antigens.

In the final study, a panel of non-microscopy diagnostics was applied to a set of banked samples from a schistosomiasis endemic area in the Democratic Republic of the Congo in order to determine whether an accurate estimate of the presence of schistosomiasis could be made when traditional microscopy is unavailable (chapter 7). Both the POC-CCA test as well as real-time PCR on stool showed to be equally suitable for a first screening of the presence of *Schistosoma* infections, whereas the UCP-LF CAA test detected many additional cases, mainly of low intensity. These results confirm that – even in the absence of microscopy – sufficient alternative diagnostic methods are available to accurately determine the presence as well as the intensity of schistosome infections in an endemic area. The POC-CCA findings, confirmed by real-time PCR, consolidate the WHO recommendation to use the POC-CCA as an alternative for microscopy in African intestinal schistosomiasis [37,64]. When more resources are available, the use of real-time PCR or the UCP-LF CAA test – provided that the UCP-LF CAA test becomes widely available – could be considered to obtain a more accurate estimation of the presence of schistosomiasis.

Reflections on the use of circulating anodic antigen detection in context-specific settings

A comparison of CAA-levels between the studies described in this thesis

The work presented in this thesis confirmed that the UCP-LF CAA test is suitable for application in both endemic and non-endemic settings. The test can be applied on non-invasive urine samples (often preferred in endemic settings) and on serum samples (more suitable for non-endemic settings). The sensitivity of the test is dependent on the test format used: by applying larger sample volumes (i.e. concentration of a pre-treated sample) a lower limit of detection can be reached (i.e. lower concentrations of CAA can be detected) [11]. Which test format is most suitable depends on the setting (low/moderate/high endemicity), the type and volume of sample available for analysis, but also on the availability of the required equipment (i.e. centrifuge) to execute the UCP-LF CAA test.

Figure 1 combines the CAA-levels as described in the different chapters of this thesis, with relevant additional details included in Table 1. In the studies where the UCP-LF CAA test was applied on both urine and serum samples (chapter 2 and 3), CAA-levels in serum tended to be higher than in urine. An observation which has also been made in previous studies [8,10,56,65-69]. CAA-levels in migrants resembled CAA-levels in individuals from endemic settings (chapter 3, 6 and 7), most likely due to repeated exposure over a longer time period in migrants. Different CAA-levels were observed in endemic settings (chapter 6 and 7), most likely depending on the level of endemicity but also on the population being measured: school-aged children in Côte d'Ivoire (chapter 6) compared to individuals of all ages in the Democratic Republic of the Congo (chapter 7). Generally, school-aged children tend to have a higher infection intensity compared to adults [70]. CAA-levels in travelers appear to be lower than those in migrants or individuals from endemic areas [9,17,21]. However, in a group of Belgian travelers relatively high CAA-levels were observed (chapter 2), suggestive of a high worm burden. This in contrast to a recent prospective study in travelers where very few individuals were positive by UCP-LF CAA while antibodies were detected in 21% of individuals [17], indicating that these travelers had a low worm burden or did not even establish an active infection.

Recommendations for using circulating antigen diagnostics in context-specific settings

In Table 2 an overview is given of diagnostic requirements and recommendations for context-specific settings. Generally, the focus in endemic settings is on ease-of-use and field applicability and costs are often crucial, whereas in non-endemic settings more advanced diagnostic methods are applied and costs usually have low priority. Early 2022, the routine diagnostics microbiology laboratory of the LUMC implemented the UCP-LF CAA test [71], making the test available for individual case detection in order to only treat those who really need it. In endemic settings, the UCP-LF CAA test could also be an ideal candidate for field use and integration into national programs, provided that the test becomes available on a large scale. The test meets the need of a highly sensitive and specific diagnostic tool – applicable to all *Schistosoma* species – for precisely mapping schistosomiasis prevalence and infection intensity at high spatial resolution to guide treatment and identify transmission hotspots.

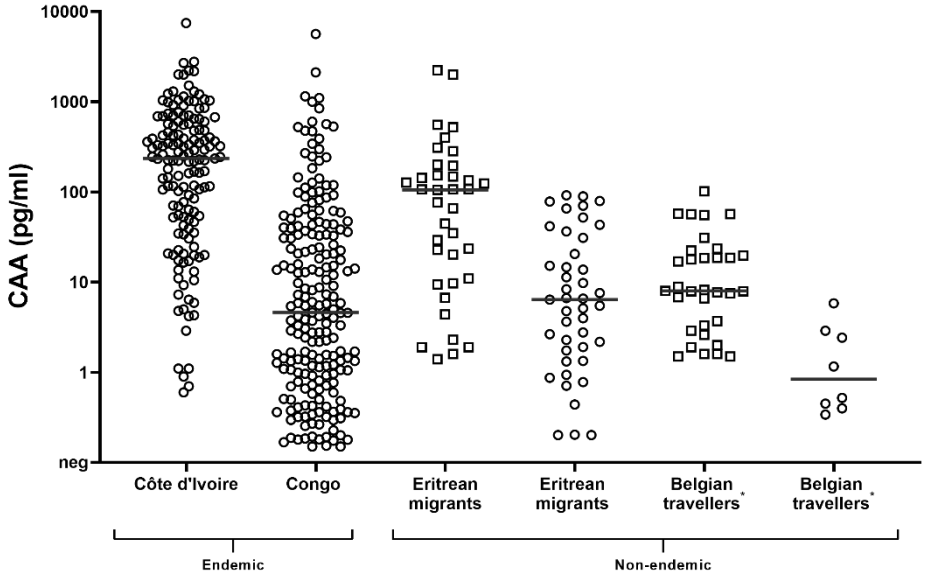

Figure 1. Overview of CAA-levels as observed in the different chapters of this thesis. Côte d'Ivoire (**chapter 6**) and Congo (**chapter 7**) are both *Schistosoma* endemic areas while the Eritrean migrants (**chapter 3**) and Belgian travelers (**chapter 2**) are representatives of non-endemic settings. CAA was measured in urine (circles) and / or serum (squares) samples. Median CAA-level (of the CAA-positives) is indicated with a horizontal red line. For more specific details see Table 1 below.
* highest observed CAA-level before PZQ treatment was used.

Table 1. Overview of additional details from the studies described in this thesis

Study (chapter)	Côte d'Ivoire (chapter 6)	Congo (chapter 7)	Eritrean migrants (chapter 3)	Eritrean migrants (chapter 3)	Belgian travellers (chapter 2)	Belgian travellers (chapter 2)
Age range study population	5-17 years	1-80 years	18-63 years	18-63 years	5-49 years	5-49 years
Species	*S. mansoni*	*S. mansoni*	*S. mansoni*	*S. mansoni*	*S. mattheei* x *S. haematobium*	S. *mattheei* x S. *haematobium*
UCP-LF CAA test format	UCAA*bT*417 wet	UCAA2000 wet	SCAA500 wet	UCAA*bT*3333 dry	SCAA500 wet	UCAA2000 dry
Sample type	urine	urine	serum	urine	serum	urine
Limit of detection	0,6 pg/ml	0,1 pg/ml	1 pg/ml	0,2 pg/ml	1 pg/ml	0,3 pg/ml
N tested	147	314	92	92	34	34
N positive	147	205	43	37	33	8
Median CAA-level of the positives	221 pg/ml	5 pg/ml	107 pg/ml	7 pg/ml	8 pg/ml	0.9 pg/ml

Table 2. Overview of recommended diagnostic methods for each context-specific setting.

	Essential characteristics				Proposed diagnostic method(s)[1]	
	Accuracy	Sample type	Ease of use	Cost of test	First choice	Optional / Alternative
ENDEMIC SETTINGS						
Control programs / Monitoring & Evaluation	Moderate	Urine, Finger prick blood	Field applicable, rapid	High priority	POC-CCA	CAA-RDT[2] Hematuria dipsticks[3]
Elimination as a public health problem	Moderate	Urine	Field applicable, rapid	High priority	POC-CCA RPA[2]	
	High	Urine	Minimal labor (central laboratory facility)	Low to moderate priority	UCP-LF CAA	Real-time PCR[4]
Interruption of transmission	High	Urine, Serum	Minimal labor (central laboratory facility)	Low to moderate priority	UCP-LF CAA	Real-time PCR[4] Antibody detection[5]
NON-ENDEMIC SETTINGS						
Travelers	High	Serum	Suitable for routine diagnosis	Low priority	UCP-LF CAA Antibody detection[5] Real-time PCR[4]	
Migrants	High	Urine, Serum	Suitable for routine diagnosis	Low priority	UCP-LF CAA Real-time PCR[4]	POC-CCA Antibody detection[5]
RESEARCH-SPECIFIC SETTINGS						
Controlled Human Infection Model	High	Urine, Serum	Highly standardized	Low priority	UCP-LF CAA Antibody detection[5,7]	
Clinical studies[6]	Moderate	Urine, Finger prick blood	Field applicable	Moderate priority	POC-CCA	CAA-RDT[2]
	High	Urine	Minimal labor (central laboratory facility)	Moderate priority	UCP-LF CAA	Real-time PCR[4]

1. Antibody detection (all *Schistosoma* spp.), PCR (all *Schistosoma* spp.), POC-CCA (*S. mansoni*), RPA (*S. haematobium*), UCP-LF CAA (all *Schistosoma* spp), CAA-RDT (all *Schistosoma* spp), Hematuria dipsticks (morbidity marker for *S. haematobium*).
2. Needs further validation.
3. Hematuria dipsticks only provide information with regard to morbidity most likely related to *S. haematobium* infection.
4. Depending on the DNA target and test format used, in addition might need collection of stool and/or urine samples.
5. Depending on the antigen target, antibody type and test format used.
6. Depending on the endemic situation, availability of resources and research question.
7. Assuming infection of schistosomiasis naïve individuals.

The high focality of schistosomiasis transmission and its dependence on a complex interplay between socio-economic, sociological (behavior of people, social situation), geographical (distance to water bodies/transmission sites) and ecological (vegetation, rainfall, occurrence of intermediate snail host) factors often result in a large variation of prevalence and intensity of infection within an area. The high accuracy and quantitative outcome of the UCP-LF CAA test, in combination with its applicability on easy and non-invasively obtained urine samples, make it amendable to pooled sample testing strategies through which information from whole communities can be obtained in a presumably more cost-effective way [72]. Compared to exhaustive individual sampling and testing approaches, appropriate pooling strategies can significantly reduce logistical and laboratory costs, with minimal loss of sensitivity and specificity [72-74]. Preliminary exercises have shown that average CAA-levels from pooled urines in defined low and high prevalence and infection intensity easily can be detected with the UCP-LF CAA test [72].

Control programs (endemic setting)

Schistosomiasis control in endemic countries relies mainly on transmission intervention measures combined with large-scale administration of PZQ without prior diagnosis, which has been successful in reducing infection intensity, and hence morbidity [38-40,75]. Programs are based on pilot surveys often performed on a limited number of school-aged children. For monitoring and evaluation of these programs and to determine whether MDA schemes should be adapted or even stopped, more sensitive non-microscopy diagnostic procedures are needed [76]. An example is the POC-CCA urine strip test, recommended as an alternative tool to Kato-Katz for mapping prevalence of African intestinal schistosomiasis as well as for surveillance purposes [37,64,77,78]. As there is no direct rapid diagnostic test for diagnosing *S. haematobium* infections commercially available, an optional method for obtaining an indication of urogenital schistosomiasis prevalence would be hematuria dipsticks that test for *S. haematobium* related microhematuria, since this strongly correlates with urogenital schistosomiasis [79]. However, although hematuria dipsticks are not expensive and relatively easy to use, they only provide information on morbidity and do not provide a confirmed schistosome-specific diagnosis of infection. A visually scored finger prick blood-based CAA rapid diagnostic test (currently under development, see below and [80-82]) could potentially be a suitable candidate for easy, quick and more accurate screening of the presence of infections with all *Schistosoma* spp.

Elimination of schistosomiasis as a public health problem (endemic setting)

In areas where morbidity has been significantly reduced, the next aim is elimination of schistosomiasis as a public health problem. This has been defined by the WHO as <1% of school-aged children with schistosomiasis being categorized as heavily infected [83]. For intestinal schistosomiasis, this means >400 eggs per gram of feces and for *S. haematobium* >50 eggs per 10ml of urine [84], detected by Kato-Katz or urine filtration, respectively. However, a recent viewpoint from Wiegand et al (2022) suggests there is not enough evidence supporting this definition and highlights the need for more accurate measurements to develop an evidence-based framework focusing on the use of overall prevalence of infection rather than the prevalence of heavy-intensity infections [85]. As the POC-CCA test has been shown to be a more sensitive and cost-effective alternative for determining *S. mansoni* prevalence, attempts

have been made to estimate equivalent measures of prevalence between POC-CCA and Kato-Katz (11,78,86-88). In addition, the diagnostic position of the potentially user-friendly recombinase polymerase amplification (RPA) assays, in particular as an alternative for the POC-CCA test in case of *S. haematobium* infections, should be further explored [89]. A visually scored finger prick blood-based CAA rapid diagnostic test (currently under development, see below and [80-82]) would also be a good alternative for accurately screening prevalence and intensity of infection with all *Schistosoma* spp.

Interruption of transmission (endemic setting)

Accurate diagnosis of schistosomiasis is also crucial for determining interruption of transmission and eventual elimination, especially in regions where extensive control measures have reduced the prevalence and intensity of infection to very low levels [3,8,68,90-92]. This is clearly recognized by the WHO and international stakeholders in the NTD Roadmap 2030 where they highlight the need for field-deployable, intelligent diagnostics and sampling strategies to evaluate pre-and post-intervention prevalence, especially for low endemic and near elimination areas. The UCP-LF CAA test has demonstrated to be suitable for determining the presence of *Schistosoma* infections in low-endemic settings [3,90,92]. Recently, a strategy for the sustained, local interruption of transmission of schistosomiasis was presented in a viewpoint paper stressing the need for highly sensitive diagnostics (e.g. the UCP-LF CAA test) and intelligent testing procedures such as pooled sampling [74]. Additionally, identifying, treating, and following-up positive cases, including those with very light intensity infections, has the added advantage of avoiding reappearance of infection that is sometimes seen in such settings due to limited cure rates and the fact that immature schistosomes are refractory to praziquantel [93]. To what extent DNA detection methods might be suitable for defining interruption of transmission, needs more research [94-96]. Eventually, in settings where transmission appears to have been interrupted, detection of specific antibodies may also play an important role, for example in assessing exposure in young children [47,97-102].

Research

The UCP-LF CAA test has been investigated, evaluated and applied in numerous studies in Africa, Asia and South America, as well as in projects in Europe and the USA, including the studies described in this thesis. Even though implementation of the UCP-LF CAA test requires a basic equipped laboratory, the current format of the test has proven to be well-developed and robust enough to be implemented in low-resource settings. This has already been realized in two clinical trials in Madagascar (*S. mansoni*) and Gabon (*S. haematobium*) within the freeBILy project, where the UCP-LF CAA test, together with the POC-CCA, is being evaluated for diagnosing and monitoring treatment of *Schistosoma* infections in the still often neglected group of pregnant women and their new born children [103-105]. Moreover, the UCP-LF CAA test has been implemented in Tanzania for HIV-*Schistosoma* coinfection and PZQ treatment studies [106-116], and more recently also in Texas (USA) and Uganda for accurate assessment of vaccine efficacy in upcoming *S. mansoni* vaccine trials [117,118].

The UCP-LF CAA test has also great potential for use in controlled human schistosome infection trials. The UCP-LF CAA test appeared to be highly suitable for monitoring cure, as demonstrated in a recent experimental human *S. mansoni* infection model, where healthy volunteers were intentionally infected with male-only or female-only parasites [10,119]. This model provided insight into the development of (acute) schistosomiasis in terms of symptoms, the related immune responses, and the performance of diagnostic tests over time. Following experimental infection, all previously schistosomiasis-naïve volunteers showed detectable antibodies against adult worm gut antigen within 4 to 6 weeks, while the UCP-LF CAA test was most suitable to determine presence as well as cure of infection after treatment. In general, post-exposure CAA-levels appeared to be relatively low (with serum concentrations ranging between 1-10 pg/ml), but after treatment decreased to below the detection limit of 1 pg/ml.

While the UCP-LF CAA test has demonstrated excellent performance, with the most sensitive concentration format in serum showing a lower limit of detection corresponding to a single worm pair in a non-human primates model [11,120], it currently does not meet the current target product profile (TPP) requirements [121]. In particular in terms of the ease-of-use and throughput, which are currently not met due to the sample pre-treatment and concentration step, improvement is needed. Furthermore, a dedicated strip reader is needed to visualize and interpret the outcome of the UCP-LF CAA test. Especially when moving to field settings, requirements for such a reader include the ease-of-use, affordability and suitability for remote settings. Efforts to make CAA detection generally available are ongoing, with a recent initiative focusing on the development of a more easy-to-use, accurate, affordable and visually scored CAA-RDT [80-82]. This CAA-RDT will be able to detect all *Schistosoma* spp in a single finger prick blood sample without the need for sample preparation or a reader for detection in order to support national schistosomiasis control and elimination programs.

Concluding remarks

This thesis has provided further evidence on the suitability of CAA detection for diagnosing *Schistosoma* infections and monitoring treatment efficacy by evaluating the UCP-LF CAA test in the context of various endemic and non-endemic settings. CAA seems to be the only diagnostic marker that is accurately detectable from the early infection stages onwards as well as cleared soon after treatment. Compared to a range of other diagnostics, the UCP-LF CAA test was confirmed to be highly sensitive and specific. Although CAA detection seems the most favorable choice overall, alternative procedures such as antibody and DNA detection methods will remain crucial for specific purposes depending on the setting. As there is no 'one size fits all', diagnostic tests for schistosomiasis need to be carefully selected based on the data they provide in order to respond adequately to a specific situation. This is the first and foremost important step after which further choices can be guided by for example practicability, test availability and costs.

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