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A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of *Schistosoma* circulating anodic antigen

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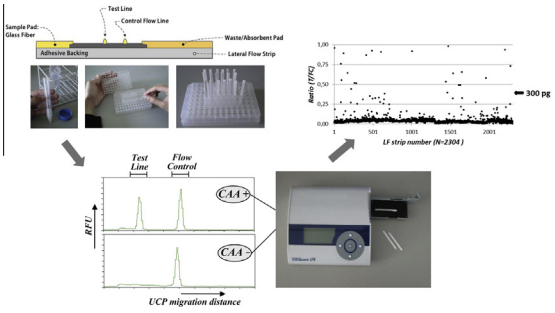
HIGHLIGHTS

- Improved lateral flow strip assay to diagnose active *Schistosoma* infection.
- Assay materials can be stored and shipped worldwide, both at ambient temperature.
- Assay materials shipped from the Netherlands, evaluated in Africa by local staff.
- The lateral flow strip assay performed as least as good as the ELISA.
- Validation of lightweight reader for analysis of the test strips.

GRAPHICAL ABSTRACT



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ABSTRACT

An earlier reported laboratory assay, performed in The Netherlands, to diagnose *Schistosoma* infections by detection of the parasite antigen CAA in serum was converted to a more user-friendly format with dry reagents. The improved assay requires less equipment and allows storage and worldwide shipping at ambient temperature. Evaluation of the new assay format was carried out by local staff at Ampath Laboratories, South Africa. The lateral flow (LF) based assay utilized fluorescent ultrasensitive up-converting phosphor (UCP) reporter particles, to be read by a portable reader (UP*link*) that was also provided to the laboratory. Over a period of 18 months, about 2000 clinical samples were analyzed prospectively in parallel with a routinely carried out CAA–ELISA. LF test results and ELISA data correlated very well at CAA concentrations above 300 pg/mL serum. At lower concentrations the UCP–LF test indicates a better performance than the ELISA. The UCP–LF strips can be stored as a permanent record as the UCP label does not fade. At the end of the 18 months testing period, LF strips were shipped back to The Netherlands where scan results obtained in South Africa were validated with different UCP scanning equipment including a novel, custom developed, small lightweight UCP strip reader (UCP-Quant), well suited for testing in low resource settings.

Conclusion: The dry format UCP–LF assay was shown to provide a robust and easy to use format for rapid testing of CAA antigen in serum. It performed at least as good as the ELISA with respect to sensitivity and specificity, and was found to be superior with respect to speed and simplicity of use. Worldwide shipping at ambient temperature of the assay reagents, and the availability of small scanners to analyze the CAA

Abbreviations: AWA, adult worm antigen; CAA, circulating anodic antigen; CCA, circulating cathodic antigen; FC, flow control; HSLF, high salt lateral flow; LF, lateral flow; MαCAA, mouse anti-CAA; POC, point-of-care; QC, quality control; SD, standard deviation; T, test; TCA, trichloroacetic acid; UCP, up-converting phosphor.

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UCP–LF strip, are two major steps towards point-of-care (POC) applications in remote and resource poor environments to accurately identify low (30 pg CAA/mL serum; equivalent to about 10 worm pairs) to heavy *Schistosoma* infections.

1. Introduction

Schistosomiasis control has recently gained increasing interest by the commitment of the 65th World Health Assembly (World Health Organization (WHO), 2012) to support interruption of transmission and even elimination is considered feasible in a number of countries. For these efforts, the availability of highly accurate diagnostics becomes a key issue and alternatives for the current standard of microscopical counting of parasite eggs in urine or stool to diagnose active schistosome infections are urgently needed. The detection of parasite-derived circulating antigens in various diagnostic assays with high sensitivity and specificity has been described extensively (Gabriel et al., 2012; Mendoza et al., 2009; Utzinger et al., 2011; van Lieshout et al., 2000; Wilson et al., 2006). Two well described circulating antigens for *Schistosoma*, both applied to diagnose active infection, are the circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA).

The circulating cathodic antigen CCA can be detected in untreated urine of individuals with active *Schistosoma mansoni* infection. After initial development of monoclonal antibody based ELISA's (de Jonge et al., 1990), a rapid point-of-care (POC) test for detection of CCA in urine has been described (van Dam et al., 2004). Several versions of this test were developed before eventually outsourcing it to Rapid Medical Diagnostics (Pretoria, South-Africa); the device has now been evaluated in various studies (Coulibaly et al., 2011; Legesse and Erko, 2007; Midzi et al., 2009; Shane et al., 2011; Standley et al., 2010). The POC–CCA test provides a rapid visual result based on a carbon or gold label but is developed for urine testing only. In the various evaluations it showed sufficiently high sensitivity and specificity to be taken up as an alternative to egg microscopy in mapping studies and field surveys. The test is particularly well-suited to accurately demonstrate moderate to heavy *S. mansoni* infections and can be considered as a useful method for *S. mansoni* diagnosis in peripheral health centers and schistosomiasis control programs (Coulibaly et al., 2011). Unfortunately, the accuracy of the POC–CCA test in *Schistosoma haematobium* infections is variable and needs to be further evaluated (Midzi et al., 2009; Obeng et al., 2008; Stothard et al., 2009).

Also for the second well-described schistosomal circulating antigen, circulating anodic antigen CAA, highly sensitive and specific monoclonal antibody based ELISA's were developed and applied in numerous epidemiological and laboratory studies (Agnew et al., 1995; Deelder et al., 1989; Leutscher et al., 2008; van Dam et al., 1996a; van Lieshout et al., 1995). CAA is a genus-specific antigen with a unique carbohydrate structure (Bergwerff et al., 1994), present in serum and urine of hosts infected with various species of *Schistosoma*, including species infecting cattle (de Bont et al., 1996; Flowers et al., 2002; Gabriel et al., 2002). The test requires a trichloroacetic acid (TCA)-precipitation step after which CAA can be detected in the supernatant. Because of this sample pretreatment procedure and the fact that only an ELISA is available for testing, detection of CAA – despite showing a much larger potential for very sensitive detection of active schistosomiasis – for many years remained a laboratory-based assay. When the test is implemented in the daily routine, with several built-in controls and samples tested in duplicate, the ELISA may detect CAA in serum at levels as low as 40 pg/mL (Leutscher et al., 2008). When the assay is performed on a less regular basis and samples are tested only once, this sensitivity may not always be reached. ELISA standard series indicate an exponential increase in signal from around 300 to 10,000 pg/mL (Corstjens et al., 2008); a concentration of 300 pg/mL seems a "safe" level to prevent unacceptable high numbers of false positives when testing under non-optimal conditions.

Schistosoma worm pairs excrete a steady amount of CAA in the bloodstream upon feeding and the day-to-day variation of CAA in serum is fairly constant implying that the time of day is irrelevant for sample collection (Polman et al., 1998). Studies on *in vitro* incubated worms as well as studies with experimentally infected animals have indicated that a single worm pair would excrete a daily amount of CAA in the order of 40 ng, corresponding to 1–10 pg/mL blood (van Dam et al., 1996a; Wilson et al., 2006). In contrast to CCA which shares Lewis-X epitopes with various host components (van Dam et al., 1996b), the CAA carbohydrate structure (repeating GalNAC and GlcA disaccharides) is completely unique and no biological equivalent has so far been described. The use of CAA specific monoclonal antibodies in combination with ultra-sensitive detection platforms could thus be expected to result in further sensitivity improvements without compromising specificity.

In order to pursue the above, and also to further improve the robustness of the CAA assay and make it more applicable for future POC applications, we recently introduced a lateral flow based platform in combination with an ultrasensitive reporter technology. The resulting LF assay demonstrated an analytical sensitivity down to 1 pg/mL, about 10-fold better than the CAA–ELISA (Corstjens et al., 2008). However, the applied format was not yet optimal for distribution because of a limited batch size and due to the fact that some of the reagents needed refrigeration. Furthermore, the requirement of a sonication step added to the complexity of the assay. Here we describe a further advance towards a field applicable test through the introduction of dry reagents. The improved field-applicable assay was tested in a routine diagnostic setting in South Africa by local staff, with dry assay materials that were shipped at ambient temperature from The Netherlands. In parallel, a custom designed lightweight reader to analyze the UCP–LF strips was tested successfully.

2. Materials and methods

2.1. Patient population and sample treatment

During a period of 18 months, 2599 serum samples were routinely analyzed for schistosomiasis by CAA–ELISA using the standard operating procedures of the Department of Serology of Ampath Laboratories (ISO 15189 and GLP/GPC certified, under supervision of Dr. L.H. van Rooyen, Dr. du Buisson, Kramer, Swart, Bouwer Inc., Centurion, South-Africa). These sera were additionally evaluated using the CAA UCP–LF strip, and after quality control, full data records of 1979 samples were obtained. The samples were sent in by local physicians, mostly from schistosome endemic regions North and East of Pretoria, based on clinical complaints and suspicion of schistosomiasis. In this population, based on extensive testing in preceding years, about 8% was expected to have active schistosomiasis, predominantly caused by