Up-Converting Phosphor Technology-Based Lateral Flow Assay for Detection of *Schistosoma* Circulating Anodic Antigen in Serum

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Schistosomiasis (reviewed in references 10 and 18) is considered one of the major helminth diseases in the world. Despite the availability of an effective drug and the implementation of successful control programs, the number of infected cases has not decreased during the last decades and is still estimated to be around 200 million people (9, 26). The main burden of disease occurs in sub-Saharan Africa, where individuals are continuously exposed to new infections while in contact with cercaria-contaminated fresh water. In countries where the disease is not endemic, schistosomiasis is considered the major imported helminth infection, found in immigrant as well as tourist travelers (1–3, 25). Although many infected cases are asymptomatic initially, long-term and heavy infections are associated with severe morbidity. Even light infections may cause serious disease, such as Katayama fever, or neurological and genital complications (10, 18).

Diagnosis of the infection is classically based on the detection of parasite eggs in urine or in feces. However, this method has several disadvantages. The number of excreted eggs is often low and shows a high day-to-day fluctuation. Therefore, stool or urine examination needs to be repeated several times. Alternatively, detection of antibodies is a highly sensitive and specific method to diagnose schistosomiasis. High antibody responses are generally seen with travelers originating from areas where schistosomiasis is not endemic. However, in immigrant travelers with a life-long history of exposure, antibody responses are mostly moderate to low. Some may even become serologically negative, while still excreting viable eggs. In addition, antibody levels are not associated with the actual worm burden and remain unaffected by treatment of the infection. Consequently, serology mostly gives straightforward answers for patients tested within months after their first exposure, but data are difficult to interpret for those who have a history of previous infection (23). A sensitive, serum- or urine-based test demonstrating active *Schistosoma* infection would be valuable in these cases.

Assays for the detection of *Schistosoma* circulating antigens (adult worm gut-associated antigens) seem very promising, as serum levels of circulating anodic antigen (CAA) are related to actual worm burden and rapidly decrease following drug treatment (reviewed by van Lieshout et al. [23]). The current monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) determines serum CAA levels for all human *Schistosoma* species with virtually 100% specificity (7); its value in (sero)epidemiological studies dealing with populations with moderate- to high-intensity infections has been demonstrated previously (17). However, the CAA-ELISA still lacks sensitivity when testing light infections, e.g., in the group of international travelers (24). Moreover, due to the relatively high complexity of the test, it lacks robustness if performed only occasionally for single case identification. This may hamper implementation of the CAA-ELISA within clinical routine diagnostic settings.

In this report we describe the development of a rapid test strip designed to detect CAA in single serum samples by utilizing the same genus-specific monoclonal antibodies as the current standard CAA-ELISA. Sensitivity of the test is increased by up-converting phosphor technology (UPT) using instrument-assisted assay analysis (5). The important advantages of UPT reporters compared to other (conventionally...
applied) fluorescent labels include high sensitivity (no auto-fluorescence of other biological materials), long shelf life, permanent record (no fading), and low costs (28). Various biosays, including lateral flow (LF) devices, that demonstrate the usefulness of UPT reporters have been developed (11, 12, 14, 15, 21, 27, 29).

We analyzed the diagnostic performance of the novel UPT-LF assay for the detection of CAA with a set of well-characterized serum samples originating from different areas of schistosomiasis endemicity, as well as a series of serum samples from schistosomiasis-suspected individuals submitted to our diagnostic laboratory for serological analysis. The latter group comprised a heterogeneous mixture of Dutch citizens (region where schistosomiasis is not endemic) that may have been infected during recent travel and immigrants with an unclear history of exposure. *Schistosoma* infections are expected to be very rare in this population. The correlation between the results of the routine antibody test, the standard CAA-ELISA, and the new UPT-LF assays was determined.

### MATERIALS AND METHODS

**CAA-specific UPT-LF strips.** A laminated nitrocellulose membrane (HiFlow Plus HF90004; Millipore Corp., Bedford, MA) was provided with a test line composed of mouse monoclonal anti-CAA antibody 147 (LUMC, Parasitology) by using a load of 175 ng antibody per 4 mm. The membrane was furthermore provided with a flow control line composed of a rabbit anti-mouse antibody (Sigma). Antibodies were diluted to 0.45 mg/ml in TM buffer (10 mM Tris, pH 8, 1% [vol/vol] methanol) and applied using a Linomat IV stripper (Camag Scientific, Inc., Muttenz, Switzerland). The striped nitrocellulose, a glass fiber sample application pad (glass 33; Schleicher & Schuell, Keene, NH), and a paper absorbent pad (filter paper 470; Schleicher & Schuell) were mounted on plastic backing and cut into 4-mm-width LF strips as described earlier (4). Strips were stored dry in containers with silica and are stable for up to a year. A schematic of the LF strip is presented in Fig. 1.

**UPT reporter conjugate.** Mouse monoclonal anti-CAA antibody 147 (LUMC, Parasitology) was coupled to 400 nm UPT reporter particles (OraSure Technologies, Inc., Bethlehem, PA) as described earlier (4), utilizing a conjugation load of 25 μg antibody per mg reporter particles. The resulting UPTM

![Diagram](https://example.com/diagram.png)

**FIG. 1.** Illustration of the UPT-LF CAA test strip and UPT-LF CAA assay. The immunoassay was performed in microtiter plate wells. After binding of the antigen to the CAA-specific UPT reporter, lateral flow was initiated by placing LF strips in the microtiter plate wells. The LF strips have a width of 4 mm and a total length of 7.8 cm and contain a CAA-specific test line and a UPT control line. Upon completion of the flow, the LF strips were interrogated with infrared light, revealing the deposition of the UPT reporter along the strip. Examples of scans obtained with positive and negative UPT test line signals are shown.

CAA concentrations were determined in serum as described previously (16). Serial dilutions of the trichloroacetic acid (TCA)-soluble fraction of *Schistosoma* adult worm antigen (AWA-TCA) were assayed simultaneously on each ELISA plate to calculate CAA concentrations. AWA-TCA contains approximately 3% (wt/wt) CAA. The lower limit of detection (LOD) of the CAA-ELISA is 10 pg CAA/ml.

**Sample pretreatment and standards.** All serum samples tested by CAA-ELISA or UPT-LF were pretreated with TCA to remove interfering proteins and to dissociate immune complexes (8). After TCA extraction, the samples were subjected to a neutralization step, resulting in a 1:4 dilution compared to the original serum. A standard serial dilution of AWA-TCA was used to determine the analytical sensitivity of the UPT-LF assay.
Characterized samples from areas of schistosomiasis endemicity. For an initial laboratory evaluation of the UPT-LF assay, four sets (10 serum samples each), representing different *Schistosoma* infection levels, were selected. These well-defined samples were collected earlier as part of epidemiological studies on the prevalence and intensity of *S. mansoni* infections. Ten low-reactive serum samples were selected from banked specimens from a study performed at Saracacca, Surinam, an area with low *S. mansoni* transmission (22). The CAA concentrations as determined by ELISA ranged from 190 pg/ml to 7.5 ng/ml (median, 1.9 ng/ml). Ten moderate- and ten high-reactive serum samples were selected from banked specimens from a study performed at Mwanza, Tanzania (13), an area with moderate to high transmission. The moderate-intensity-infection set had CAA concentrations ranging from 3.5 to 28.5 ng CAA/ml (median, 11.1 ng/ml), whereas in the high-intensity-infection set the CAA concentrations ranged from 3.45 to 114.0 ng CAA/ml (median, 66.2 ng/ml). The specificity of the assay was tested with 10 serum samples selected from banked specimens from a study performed in an area of Senegal where schistosomiasis is not endemic (16). No *Schistosoma* eggs were found in the latter group following extensive stool and urine examination, and all samples tested negative in the CAA-ELISA.

Schistosomiasis-suspect samples from areas of schistosomiasis nonendemicity. A second evaluation study of the UPT-LF assay was performed with 166 serum samples sent to our reference laboratory by general practitioners or other diagnostic laboratories within The Netherlands. All samples originated from individuals living within The Netherlands and were sent with the request of Schistosoma antibody testing because of suspected schistosomiasis. This group represents recent travelers or immigrants from a region of schistosomiasis endemicity. Routine testing implied screening of serum by two “in-house” antibody assays, an immunofluorescence assay for anti-adult worm antibodies (IFA-AWA) and an ELISA for anti-soluble egg antigen antibodies (ELISA-SEA) (23). IFA-AWA titers of $\geq 16$ and ELISA-SEA titers of $\geq 32$ were considered positive. Based on serology outcome, the group was divided into an antibody-negative group ($n = 43$) and an antibody-positive group ($n = 123$). Samples were tested in the CAA-ELISA for comparison with the UPT-LF assay.

RESULTS

Analytical sensitivity of the UPT-LF assay. The analytical sensitivity of the UPT-LF assay was determined by analyzing an AWA-TCA dilution series. The result of a typical experiment with direct comparison of the UPT-LF assay and the CAA-ELISA ($n = 6$) is shown in Fig. 2A. In this set of experiments, the UPT value obtained with 1 pg/ml CAA was well above the zero control. A more detailed analysis (Fig. 2B) in the lower concentration range demonstrated that the UPT-LF assay was able to detect 0.5 pg/ml CAA (lowest concentration tested). The UPT-LF value obtained at this concentration was 0.083, relevantly higher then the 0.038 value obtained with the zero control.

Cutoff threshold value for serum samples. UPT-LF tests performed with the normal human sera (negative samples obtained from 30 Dutch blood bank donors) resulted in UPT values ranging from 0.027 to 0.064 (median, 0.034), with an average of 0.036 and a standard deviation (SD) of 0.0087. The cutoff threshold above which a sample was designated “CAA positive” was set to $\geq 0.081$ (highest negative plus 2 SD), and the cutoff threshold below which a sample was designated “CAA negative” was set to $\leq 0.053$ (average plus 2 SD). Samples resulting in ratio values between 0.053 and 0.081 were designated “potentially positive.” The positions of the positive and negative cutoff thresholds determined with TCA extracted sera are indicated in Fig. 2B.

Evaluation of UPT-LF with a set of characterized samples from areas of schistosomiasis endemicity. An evaluation of the UPT-LF assay with a set of 40 characterized epidemiological serum samples indicated excellent correlation with infection status as determined by the CAA-ELISA (Fig. 3). The UPT values of the 10 samples from the Surinam area of low schis-
TABLE 1. UPT-LF and ELISA analysis of 166 clinical serum samples suspected of schistosomiasis
d | UPT result | No. of serology-positive samples | No. of serology-negative samples | Total |
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<td>positive by CAA-ELISA</td>
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<td>15</td>
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<td>Potentially positive</td>
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<td>Negative</td>
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| Total      | 35               | 35              | 70   |

* Samples were divided in two groups according to their antibody responses (123 serology-positive samples and 43 serology-negative samples). A UPT value of ≥0.081 was considered positive, and a UPT value of ≥0.053 was considered negative. IFA-SEA titers of ≥16 and/or ELISA-SEA titers of ≥32 were considered positive. Samples with ≥40 pg CAA/ml were considered positive by CAA-ELISA.

tosomiasis endemicity ranged from 0.30 to 3.3 (median, 2.1). The lowest measured signal within this group was far above the positive cutoff threshold of 0.081. The sera from Tanzania with moderate and high intensities of infection ranged in UPT value from 3.0 to 5.8 (median, 4.7) and from 3.5 to 6.4 (median, 4.8), respectively. The UPT value of the Schistosoma-negative control group from an area of schistosomiasis endemicity in Senegal ranged from 0.010 to 0.026 (median, 0.015), well below the negative cutoff threshold of 0.053.

Analysis of schistosomiasis-suspect samples from an area of schistosomiasis nonendemicity. The performance of UPT-LF was furthermore investigated with 166 clinical samples submitted to our clinical reference laboratory because of suspected schistosomiasis. Their UPT values are also depicted in Fig. 3 (antibody positives and antibody negatives). The UPT-LF assay identified 36 samples, 17 being potentially positive, with UPT values ranging from 0.054 to 0.080 (median, 0.066), and 19 with unambiguously positive UPT values, ranging from 0.086 to 2.4 (median, 0.42). The remaining 130 samples scored UPT-LF values below the negative cutoff threshold of 0.053, ranging from 0.009 to 0.052 (median, 0.032). The UPT-LF results are presented in Table 1 in comparison with the CAA-ELISA results and outcome of the antibody screening. The 43 antibody-negative samples were negative in the CAA-ELISA and the UPT-LF assay, except for one potentially positive sample (ratio, 0.066). In the 123 antibody-positive cases, antigen was demonstrated by the CAA-ELISA in 15 samples (12.2%), compared to 19 (15.4%) clearly positive and 16 (13.0%) potentially positive samples in the UPT-LF assay.

DISCUSSION

CAA excreted by adult *Schistosoma* parasites is cleared rapidly from human circulation, and the concentration in serum is correlated to the intensity of the infection (23). The presence of CAA in serum as detected by the CAA-ELISA therefore is a valuable tool to diagnose active infection of *Schistosoma*. However, the current ELISA does not meet the sensitivity level to effectively diagnose imported schistosomiasis cases in countries where schistosomiasis is not endemic; these cases for a large part represent very light infections (24). Also, implementation of the ELISA in routine clinical diagnostics is complex as ELISA may lose robustness when performed only occasionally. Moreover, the ELISA was not developed for single case identification.

Recently a rapid immunochromatography test strip for the detection of *Schistosoma* circulating cathodic antigen in urine has been introduced. This simple-to-use field test has shown its value in epidemiological surveys, particularly in remote areas of *S. mansoni* endemicity (19, 20). The applied immunochromatography or LF format is suitable for single case diagnosis. In this paper, we present a UPT-LF assay for detection of CAA that was initially designed for lab-based applications. Serum assays are appropriate and readily available for this setting, whereas CAA detection in general has a higher sensitivity and specificity than circulating cathodic antigen detection (16). The UPT-LF assay, as with the ELISA, is performed on TCA extracted serum samples and utilizes the same mouse monoclonal antibody to build a CAA immunosandwich (7). The ELISA detection format is replaced with an LF-based detection of CAA-bound UPT-reporter particles.

Analytical sensitivity: comparison of UPT-LF with ELISA. The analytical sensitivity of UPT-LF indicated an LOD of 0.5 pg CAA per ml, more than 10-fold better than the 10 pg/ml for the ELISA. Assuming a CAA molecular mass between 10 and 100 kDa and a 10-μl sample volume, this translates to a detection level between 10⁶ and 10⁵ target molecules per LF strip (in agreement with LODs as determined in other UPT-LF assays [5, 29]).

The dynamic range of the UPT-LF assay is 4 orders of magnitude, spanning CAA concentrations of 0.5 pg/ml through 500 pg/ml. The 0.5-pg/ml value represents the LOD of the UPT-LF assay whereas 500 pg/ml indicates a UPT plateau value. It is possible to perform quantitative measurements above 500 pg/ml by increasing the number of UPT reporter particles in the assay; this, however, negatively affects the LOD. In the very few cases (in regions where schistosomiasis is not endemic) that UPT-LF diagnosis indicates concentrations above 500 pg/ml and further quantification is required, e.g., for determination of accurate drug treatment, the ELISA is applicable up to concentrations of 30,000 pg/ml. Alternatively, the UPT-LF analysis could be repeated simply with a dilution of the TCA-treated sample.

The UPT-LF cutoff threshold value used in this study was determined from samples obtained from 30 Dutch blood bank donors. Although UPT values were presented as ratio values (allowing interassay comparison [4]), an indeterminate group (potentially positive) was included because assays were performed with different batches of LF strips. All LF strips were produced manually in small batches of 40 strips. The average UPT value of 0.036 (median, 0.034) with an SD of 0.0087, determined for the blood bank donors, indicates that a negative cutoff threshold of 0.053 is not unrealistic. When using this 0.053 threshold, only one of the 30 blood bank samples and one of the serology-negative samples scored CAA positive. A precise assay cutoff threshold needs to be determined in future with a higher number of negative controls (from regions of schistosomiasis endemicity as well as nonendemicity) by using strips from large production batches.

Evaluation of the UPT-LF assay. A retrospective analysis of four defined sets of epidemiological samples demonstrated 100% diagnostic agreement between the CAA-ELISA and the
UPT-LF. For the three Schistosoma-positive serum sets, this result was not surprising, as all samples were selected based on CAA-ELISA results and the UPT-LF assay obviously demonstrated a better analytical sensitivity. Although the UPT-LF assay initially was not designed to function as a quantitative assay, excellent correlations were seen in these three serum sets between the UPT ratio and the serum CAA concentration as determined by the CAA-ELISA. Only the differentiation between moderate- and high-intensity-infection sets was less pronounced with the UPT-LF assay than with the CAA-ELISA. This is a consequence of reaching a plateau value in the UPT ratio above 500 pg CAA per ml. In addition, the specificity of the UPT-LF assay was found to be high, as samples collected in a region of Senegal where schistosomiasis is not endemic all tested clearly negative.

The performance of the UPT-LF assay was analyzed in 166 serum samples referred to our diagnostic laboratory because of suspected schistosomiasis. These samples were tested routinely by antibody serology, which is the standard procedure to identify a Schistosoma infection in a setting where schistosomiasis is not endemic (25). The UPT-LF assay identified 27% more CAA-positive samples than the ELISA and 140% more cases when including the potentially positive group. With respect to the results obtained with the negative controls, the majority of the samples from the potentially positive group are likely to be truly positive. Similarly to previous publications indicating antibody detection to be a poor indicator of an active Schistosoma infection (25), UPT values did not correlate with absolute serology values. Although more active cases were identified by the CAA-ELISA results, the majority of antibody-positive individuals had no detectable CAA levels. Based on previous data, there is no indication that lack of detectable CAA serum levels is related to the production (large batches), it can be concluded that the UPT-LF assay is of potential value in laboratory diagnosis of Schistosoma infections.

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REFERENCES


