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Correlation of serum and dried blood spot results for quantitation of *Schistosoma* circulating anodic antigen: A proof of principle

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ABSTRACT

Circulating anodic antigen (CAA) testing is a powerful, increasingly-used tool for diagnosis of active schistosome infection. We sought to determine the feasibility and reliability of measuring CAA in blood spots collected on Whatman 903 protein saver cards, which are the predominant filter papers used worldwide for dried blood spot (DBS) research and clinical care.

CAA was eluted from blood spots collected from 19 individuals onto Whatman 903 cards in Mwanza, Tanzania, and the assay was optimized to achieve CAA ratios comparable to those obtained from the spots' corresponding serum samples. The optimized assay was then used to determine the correlation of serum samples ($n = 16$) with DBS from cards that had been stored for 8 years at ambient temperature. Using a DBS volume equivalent to approximately four times the quantity of serum, CAA testing in DBS had a sensitivity of 76% and a specificity of 79% compared to CAA testing in serum. CAA testing was reliable in samples eluted from Whatman 903 cards that had been stored for 8 years at ambient temperature. The overall kappa coefficient was 0.53 (standard error 0.17, $p < 0.001$).

We conclude that CAA can be reliably and accurately measured in DBS collected onto the filter paper that is most commonly used for clinical care and research, and that can be stored from prolonged periods of time. This finding opens new avenues for future work among more than 700 million individuals living in areas worldwide in which schistosomes are endemic.

1. Introduction

The schistosome circulating anodic antigen (CAA) assay is a test of high importance for both the estimated 260 million individuals worldwide with schistosome infection, as well as the 700 million who live in endemic areas and are at risk of schistosome acquisition (World Health Organization, 2015, 2013). CAA is a glycoprotein produced in the gut of schistosome worms (De Water et al., 1986) that is secreted into the host bloodstream during active schistosome infection. CAA correlates closely with infection status, making the test useful not only for diagnosis but also for monitoring treatment response (De Jonge et al., 1989; Kremsner et al., 1994; Van Dam et al., 1996; Van Lieshout et al., 1991).

Compared to the traditional diagnosis of schistosomiasis by microscopic examination for eggs in multiple urine and stool samples, schistosome antigen testing offers advantages of single sample collection, elimination of labor-intensive work with excrement, and enhanced sensitivity with the potential to detect as little as one worm pair (Corstjens et al., 2014). Antigen testing has also been recommended for serologic screening programs in which repeated examinations of urine and stool are logistically not possible (Van Lieshout et al., 2000) and for diagnosis of young children, from whom obtaining urine and stool samples is difficult (Stothard et al., 2011). Given that the CAA assay has recently been developed into a dry-reagent lateral flow assay with a portable reader that can be easily transported to, and operated in, resource-limited settings (Van Dam et al., 2013), its utilization will likely continue to increase.

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From a laboratory standpoint, the CAA assay is an appealing test. The antigen's unique carbohydrate structure has no known biological equivalent (Bergwerff et al., 1994; Van Dam et al., 2013), and recent modifications make the assay highly sensitive (Van Dam et al., 2015). CAA is heat-resistant and extremely stable, remaining detectable in tissue isolated from Egyptian mummies (Miller et al., 1992). While this would suggest that CAA might be easily measured in dried blood spot (DBS) samples, two early studies that explored this issue in several types of filter paper have shown that CAA was detectable but that available concentrations were low (Jamaly et al., 1997; Nilsson et al., 2001). Of note, these studies did not evaluate Whatman 903 protein saver cards, which cost approximately USD $1.50 each and are the most commonly-used filter papers for HIV testing and monitoring worldwide, including early infant HIV diagnosis and HIV drug resistance genotyping (Masciotra et al., 2012; Rottinghaus et al., 2013). Whatman 903 cards have additionally been validated for detection of malaria gametocyte RNA by qRT-PCR (Jones et al., 2012; Mlambo et al., 2008). Given that numerous projects currently collect DBS from regions in which schistosomiasis, HIV, and malaria are co-endemic and that the ability to test these DBS for schistosomiasis would be useful for future patient care and research, we sought to determine the feasibility and reliability of measuring CAA in DBS on Whatman 903 cards, as compared to serum, from patients in Tanzania where all three infections are co-endemic.

2. Materials and methods

2.1. Study site

Samples for this study were collected in the Kisesa ward in northwest Tanzania, located approximately 20 km east of Mwanza city. We have previously demonstrated that the prevalence of schistosomiasis by CAA in serum is ~50% among adult women in this region near Lake Victoria (Downs et al., 2012), with a similar prevalence in adult men (unpublished data). Urine and stool microscopy demonstrated that the predominant species in the region is *Schistosoma mansoni*, with approximately 25% of community-based participants in prior studies having *S. mansoni* ova visualized in stool and approximately 2–3% having *Schistosoma haematobium* ova visualized in urine.

2.2. Sample collection for assay optimization

In April 2012, we invited women of childbearing age who were seeking care for themselves or their children at the Kisesa Health Centre to participate in this study. Four milliliters of blood were collected by venipuncture from the antecubital fossa and five spots of blood (each ~13 mm in diameter) were collected by fingerstick lancet onto Whatman 903 protein saver cards (GE Healthcare Life Sciences, Piscataway, NJ, USA). Cards were dried away from direct sunlight, placed into individual zip bags 24 h after collection, and stored at room temperature until processing. Venous blood was centrifuged upon return to the National Institute for Medical Research Laboratory in Mwanza City approximately 20 km away, and serum was stored at −20C. All women were given empiric praziquantel (40 mg/kg) in accordance with World Health Organization guidelines (World Health Organization, 2006).

2.3. Dried blood spot sample preparation

To elute dried samples from cards, we cut sections from the DBS and placed them into eppendorfs containing 100 μL of phosphate-buffered saline. The sections were incubated overnight at 4C, then placed on a shaker for 1 h at 37C, after which 100 μL of 4% (w/v) tricholoroacetic acid (TCA) was added and the mixture vortexed and centrifuged. We first eluted a 24 mm^2 DBS into a total volume of 200 μL. In order to increase the sensitivity, we subsequently eluted DBS with a total area of 144 mm^2 into final concentration of 2% TCA. The supernatant from these elutions was concentrated to a final volume of 20–30 μL using a 10 kDa concentration device (Amicon Ultra −0.5 ml Centrifugal Filters, Millipore Corp.).

2.4. CAA test strip preparation and testing

Serum samples (20 μL) were mixed with an equal volume of 4% TCA, vortexed, and centrifuged. Twenty microliters of this supernatant, 20 μL of the supernatant of the eluted samples from the small circles, or all of the concentrated eluate supernatants were subsequently mixed with assay buffer containing UCP reporter particles labelled with anti-CAA McAb, incubated, and applied to CAA-specific lateral flow test strips as previously described (Corstjens et al., 2008; 2014; Van Dam et al., 2013). Strips from serum samples were prepared at the local laboratory in Mwanza, Tanzania and then shipped with DBS to Leiden University Medical Centre for subsequent testing. Elutions, CAA strip preparation for eluted samples, and CAA strip scanning using a modified Packard FluoroCount meter were performed at Leiden University Medical Centre. The test line signal was normalized to the control line signal for each individual sample. Standards of known concentrations, run with each assay, were used to construct a standard curve and to determine the cut-off point above which samples were considered positive, which corresponded with 30 pg/ml.

2.5. Additional testing in banked dried blood spots

Following optimization of the assay, we obtained additional paired serum and DBS samples from the same Kisesa region that had been collected onto the same Whatman 903 cards from adults aged 15–49 years in 2005. The cards had been stored with desiccant packs in separate zip bags at room temperature. CAA strips from serum samples were prepared in Mwanza; DBS elution and subsequent processing were performed in the Netherlands. Elutions were performed using 216 mm^2 of dried sample and 600 μl PBS as described above, with the subsequent addition of 138 μl of 12% TCA (resulting in a final 2% (w/v)). The total volume of supernatant was concentrated to 20–30 μl.

2.6. Statistical analysis

Results were entered into Microsoft Excel and analyzed using Stata/IC Version 13 (College Station, TX, USA). Correlation between log-transformed CAA concentrations was determined using Pearson's correlation coefficient. Agreement between positive and negative test results was assessed using Cohen's kappa coefficient.

2.7. Ethics

This project was approved by the joint Bugando Medical Centre/CUHAS Research and Publications Committee, the National Institute for Medical Research in Tanzania, and Weill Cornell Medical College. Study participants provided informed consent for their participation.

3. Results

3.1. Available samples

Paired serum and DBS were available from a total of 35 individuals. Nineteen participants provided fresh blood and DBS in April 2012, which were used for test optimization between April and July