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A Rapid Point-of-Care Assay for Cysticercosis Antigen Detection in Urine Samples

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Abstract. We report a proof-of-concept study using a dipstick assay to detect *Taenia solium* antigen in urine samples of 30 patients with subarachnoid neurocysticercosis and 10 healthy control subjects. Strips were read in blind by two readers. The assay detected antigen in 29 of 30 cases and was negative in all 10 control samples. Although this study was performed in samples from individuals with subarachnoid neurocysticercosis who likely had high circulating antigen levels, it provides the proof of concept for a functional urine antigen point-of-care assay that detects viable cysts. Such an assay could serve to support a clinical diagnosis of suspect neurocysticercosis or to identify patients at risk of developing severe disease in areas where medical resources are limited, providing evidence to refer these individuals for imaging and specialized care as needed.

BACKGROUND

Neurocysticercosis (NCC) is a disease caused by invasion of *Taenia solium* larvae into the central nervous system (CNS). Neurocysticercosis is the most important cause of acquired epilepsy worldwide and contributes to other neurological morbidity as well.[1,2] *Taenia solium* is endemic to Latin America, sub-Saharan Africa, and vast parts of Asia, including China and the Indian subcontinent.[1,2] Diagnosis of human NCC relies on neuroimaging using computed tomography or magnetic resonance imaging, which are not readily available in rural endemic regions.[3,4]

Immunoassays play a significant role in supporting the imaging diagnosis, particularly in the common scenario where imaging findings are not conclusive. Antibody detection by lentil-lectin glycoprotein enzyme-linked immunoelectrotransfer blot (LLGP-EITB) is the assay of choice because of its high sensitivity and specificity, but the presence of specific antibodies can reflect exposure or prior resolved infections. Detection of circulating parasite antigen using monoclonal antibody–based ELISA is less sensitive than LLGP-EITB but has the advantage of demonstrating the presence of viable parasitic larvae, which can guide patient management.[5]

In community settings, immunodiagnosis could serve to support clinical diagnosis of suspected NCC or to identify individuals at risk of developing symptomatic disease so they can be triaged for imaging and specialized care.[6] Although blood sample collection is invasive and often refused by patients or study participants, we and others have demonstrated the presence of parasitic antigen in urine, which creates the potential for non-invasive sampling.[7,8] Recently, our group has been working on assessing the likelihood of detecting NCC by immunodiagnosis in urine samples, including ELISA antigen detection in field conditions[9] and DNA detection in patients in hospital settings.[10] Here we report the successful proof-of-concept testing of a rapid dipstick assay that was able to detect circulating cysticercosis antigen in urine samples of patients with subarachnoid NCC.

METHODS

Assay development. We sprayed 0.1 μL/mm of 3 mg/mL monoclonal antibodies against cysticercus (TsW8 McAb) in phosphate buffer (pH 7.2) onto a nitrocellulose membrane on a backing card as the test line. For the control line, 0.5 mg/mL biotin–bovine serum albumin (BSA) (catalog no. CGBIO-0600; Arista Biologicals, Allentown, PA) in phosphate buffer (pH 7.2) was sprayed at 0.1 μL/mm. The membrane was dried overnight at room temperature inside a chamber with relative humidity $< 30\%$. The absorbent pad was pasted on the backing card, overlapping the nitrocellulose membrane, and the card was cut into 3.5-mm-wide strips that were stored at room temperature in a dry box until use. Two conjugates were used: a test line detector conjugate of TsW5 McAb labeled with blue latex nanoparticles (catalog no. ab269892; AbCam, Cambridge, UK) and a control line detector of streptavidin-gold OD50 (catalog no. CGSTV-0600; Arista Biologicals, Allentown, PA). Coupling TsW5 McAb to blue latex nanoparticles followed the manufacturer's protocol. Briefly, TsW5 McAb was diluted to 0.1 mg/mL with reaction buffer A. Forty microliters of the 0.1 mg/mL antibody was added to the 500-nm blue latex vial, and nanoparticles were reconstituted by gently mixing. After incubation for 15 minutes at room temperature, coupling was quenched by adding 1 mL of 1X quencher. Five minutes later, the mixture was transferred to a microcentrifuge tube and spun at 10,000 rpm for 9 minutes. Approximately 850 μL of the supernatant was removed, and, without resuspending the pellet, the mixture was spun for 1 more minute at 10,000 rpm. The remaining supernatant was removed, and the pellet was gently resuspended in 40 μL of resuspension buffer (phosphate-buffered saline [PBS] 1× [pH 7.2] with sodium azide 0.05%) + 0.1% BSA. Conjugate labeled with blue latex was stored at –4°C until use. Optimal assay conditions were defined and standardized by adjusting concentrations and volumes of conjugates and blocking buffer (PBS Tween 0.3%, milk 1–5%, and BSA 1–5%), sample volumes, and reaction times using urine samples serial

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dilution curves from pools of well-defined cysticercosis-positive and cysticercosis-negative individuals.

Samples. We selected urine samples from 30 consecutive patients with subarachnoid NCC. Samples were anonymized and assigned consecutive numbers for processing, reading, and analysis. In addition, 10 negative urine controls were collected from adult healthy volunteers who had no known medical conditions. This sample size provided sufficient precision for proof-of-concept (95% CI = 78–99% around expected sensitivity of 95%). Samples were kept at −20°C until processing.

Processing. Samples were processed by incubating each strip with 80 μL of PBS-Tween 0.3%, 0.5 μL of gold conjugate, 1 μL of TsW5 blue latex conjugate, and 20 μL of unconcentrated urine sample in a well of a flat-bottom ELISA plate (Nunc Maxisorp; Thermo Fisher Scientific, Waltham, MA) at room temperature for 15 minutes. Target antigens were captured by TsW8 McAbs impregnated in the test line and were recognized by TsW5 McAb labeled with blue latex microspheres, developing a blue line. Gold conjugate passed through the test line and bound to the protein of the control line, coloring it red. The test was read after 15 minutes, and the result was recorded in a photographic register for future readings. Reading was performed by two different readers masked to participant diagnosis, using an arbitrary scale for test line intensities ranging from 1+ to 3+++. Additionally, all urine samples were tested by our in-house Ag-ELISA using the same pair of McAbs (TsW8/TsW5) to obtain antigenic ratios, calculated by dividing the optical density of the sample by a cutoff (mean value of several negative samples plus 3 SD).

Human subjects. This study used anonymized urine samples collected from prior diagnostic studies. All samples had been obtained under study protocols reviewed and approved by the Institutional Review Board (IRB) of Universidad Peruana Cayetano Heredia and/or the IRB of the Instituto Nacional de Ciencias Neurologicas. Participants consented to future use of samples in other diagnostic studies.

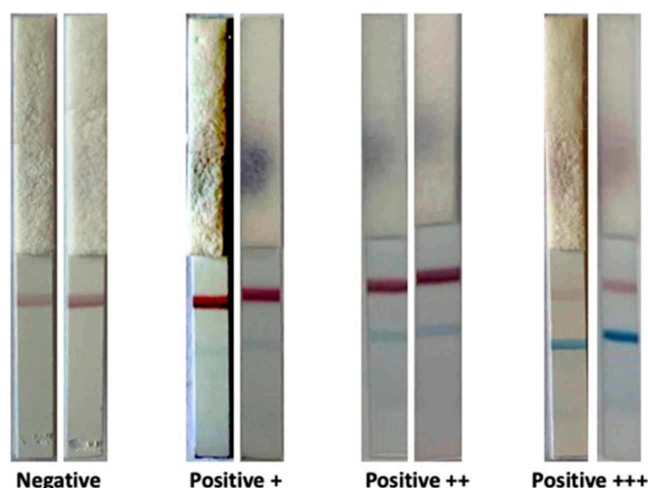

FIGURE 1. Urine antigen detection by rapid test showing representative strips for each intensity category. Red line: control line. Blue line: test line.

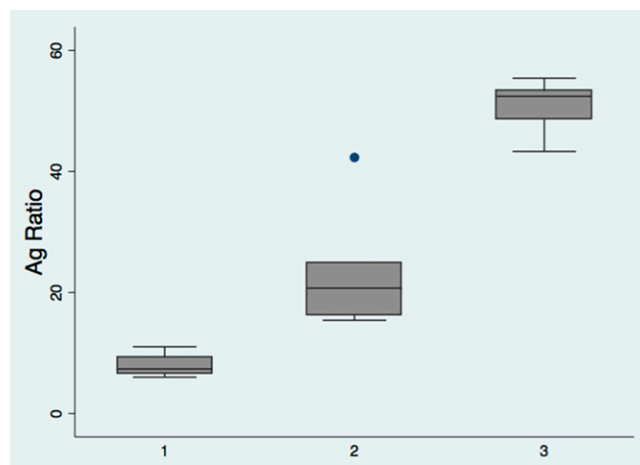

FIGURE 2. Boxplot of antigen ratio (Ag ratio) and intensity of the bands in the dipstick. 1 (+), 2 (++), and 3 (+++).

RESULTS

Median ages of participants were 50 years for cases and 47.5 years for controls; the corresponding proportions of male and female participants were 36.67 and 30%, respectively. Twenty-nine out of 30 urine samples from individuals with subarachnoid NCC (sensitivity = 97%; 95% CI = 83–100%) and none of the 10 healthy control samples (specificity = 100%; 95% CI = 69–100%) tested positive. Interrater agreement was 97.5%; readings were discordant in only one sample, which was then resolved by consensus between both readers.

Most positive samples (19/29, 66%) were read as 3+++, three (10%) as 2++, and seven (24% as 1+ (Figure 1). Antigenic ratios of subarachnoid NCC urine samples ranged from 3.4 to 55.0, on ELISA with very strong correlation with the intensity of the test line (Spearman's correlation = 0.951) (Figure 2).

DISCUSSION

We present proof-of-concept results for a functional, rapid point-of-care (POC) test to detect circulating *T. solium* antigens in the urine of patients with subarachnoid NCC. The test correctly identified as positive all but one of 30 tested case samples and was negative in all control urine samples. In addition, test band intensity was strongly correlated with urine levels as measured by standard ELISA. This represents an important first step in developing a much-needed POC assay that provides rapid results using a non-invasive sample to support diagnosis and management decisions in NCC.

The clinical presentation of NCC depends on cyst localization, numbers and volume of cysts, and the immune response of the host. Subarachnoid NCC, the most severe form of the disease, involves proliferation of the parasitic membranes leading to mass effect and chronic arachnoiditis. It is associated with hydrocephalus and intracranial hypertension and results in high mortality rates if not recognized and treated appropriately.[11] We used urine from patients with subarachnoid NCC for the initial testing phase

of this prototype dipstick because of the consistently high levels of parasite antigens characteristic of subarachnoid NCC. Clearly, this preliminary series does not assess the performance of the test in other types of NCC, such as parenchymal NCC, in which antigen levels may be much lower and test sensitivity could decrease significantly. However, even if the assay only detects high antigen levels, it could be used in the community settings to detect subarachnoid NCC in asymptomatic individuals, allowing for early diagnosis and potentially much less morbidity as well as better response to antiparasitic treatment.[9]

The test is based on two anti–*T. solium* IgM monoclonal antibodies (TsW8 and TsW5), which were produced against whole parasite extracts and previously tested in a standard ELISA.[12] Other antigen detection assays in cysticercosis rely on monoclonal antibodies to the related tapeworm *Taenia saginata*, which cross-react with *T. solium*.[5] None of the target antigens recognized by existing McAbs has been specifically identified. Because ELISAs require a well-equipped laboratory and trained operators, these assays are not available in most rural regions where this disease is endemic. Providing a quick and easy POC test could significantly change screenings and diagnosis paradigms for NCC, allowing diagnosis and management in early disease stages. Using a urine sample collected through noninvasive sampling is convenient and painless, improving population acceptability and compliance.

This POC test has potential limitations. The test recognizes circulating antigens in urine but cannot discriminate cyst location. Positive results could potentially occur in rare cases in which cysts develop in skeletal muscle rather than in the CNS. In addition, the possibility of cross-reactions with other helminths needs to be assessed.

In this proof-of-concept study, we developed a dipstick assay to detect urinary *T. solium* antigens and explored its performance for the diagnosis of subarachnoid NCC. Due to the high prevalence of NCC in endemic communities and the substantial mortality associated with subarachnoid NCC, our results suggest this rapid test could be applied to identify subarachnoid NCC cases for early intervention and specialized care in community settings or for rapid diagnostic confirmation of suspected cases in clinical settings.

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