Development and Evaluation of an Updated LATE-PCR Dipstick for the Detection of Schistosomiasis Mansoni Infection in Human Stool Sample

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***Abstract*—Schistosomiasis caused by water related trematode Schistosoma mansoni is a serious public health problem in tropical and subtropical countries. It is associated with considerable morbidity and mortality in developing and underdeveloped countries. This study aims to develop Linear-After-The-Exponential- Polymerase Chain Reaction (LATE-PCR) dipstick for the detection of Schistosoma mansoni in stool samples. In an effort to establish and enhance accurate diagnosis of Schistosoma mansoni infection, LATE-PCR dipstick was used to detect the parasite in stool samples due to the test being highly sensitive and specific, relatively simple, rapid, easy to perform. Primers and probes targeting for gene cytochrome oxidase were designed for species specific amplification of Schistosoma mansoni. In this study, the LATE-PCR dipstick parameters were optimized and detection of PCR products was performed on 2% agarose gel electrophoresis and the nitrocellulose membrane was coated with biotinylated anti-mouse IgG (control line), anti-FITC (target line) and assembled as lateral flow strips. The high sensitivity enabled detection of parasite DNA in faecal samples containing as low as 1 ng/µl of parasite DNA in faeces. The amplification reaction showed to be specific without any cross reaction with DNA from other intestinal micro-organism. The findings of LATE-PCR dipstick developed in this study may constitute a valuable alternative for the diagnosis of Schistosoma mansoni infection in underdeveloped as well as developing countries.**

***Index Terms*—LATE-PCR, lateral flow assay, Schistosoma mansoni, stool sample**

I. Background

Schistosomiasis or Bilharzia also known to many local as snail water fever, is a water-related trematode infection caused by schistosoma species, which remains a public health problem in tropical and subtropical countries of the world [1], [2], [3]. It is currently estimated that among people infected 90% are from sub-saharan Africa where schistosomiasis infections are said to be prevalence [4]. The disease is due to the eggs of small, thread-like parasitic worms living inside the blood vessels of the liver, gut and even bladder.

World Health Organization estimate of the disease epidemiology, indicate that 235 million cases of schistosomiasis occur world -wide, with 732 million people at risk of infection and another 200,000 people died each year especially in sub-Saharan Africa where the disease is said to be endemic [5][6]. According to estimate of WHO report in 2007, that between 391 and 587 million people have active cases of schistosomiasis. world-wide and that 1.7 to 4.5 million loss per annum of disability adjusted life years are due to schistosomiasis [5] [7] [6].

Many of the diagnostic techniques which exist currently have lot of limitations and are not easily practicable in the field probably due to requirements of sophisticated equipment and reagents. Performing these techniques even in the laboratory takes a lot of time and sometimes enzyme reagents involved require of a cold chain for transportation and a few steps overnight incubation may be needed [8].

Manuscript received October 28, 2014; revised September 21, 2015.

doi: 10.18178/jomb.5.2.124-127

The detection limit in light intensity infections by stool microscopy is much lower. This is because of insensitivity of microscopy and therefore prevalence in endemic areas may not be detected in stool samples accordingly [9]. In view of these problems, there is need to develop simple, sensitive and specific PCR base assay methods for screening stool samples in order to supplement microscopy.

Positive and Negative stool samples Fig. 1 and Fig. 2.

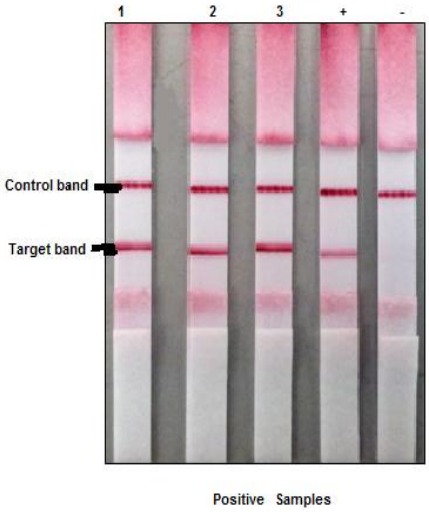

Figure 1. Positive stool samples

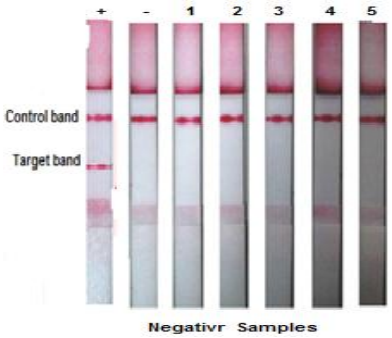

Figure 2. Negative stool samples

II. Materials and Methods

A. Materials

Antibodies used: Control Line: Anti-mouse IgG-biotin from Sigma Aldrich (St. Louis, USA)
Target Line: rabbit anti-digoxigenin IgG from Sigma Aldrich (St. Louis, USA)
Lateral flow buffer: 10 mM Phosphate Buffered Saline (pH 7.4),1% Bovine serum albumin ,0.5% Tween-20
Gold-conjugate buffer: 2 mM borate, 1% bovine serum albumin, 5% trehalose

B. Test Sample

A total of 30 samples were collected from schistosomiasis positive patients by parasitological method. A stool sample was obtained and DNA was isolated using commercial DNA kit. Twenty apparently healthy subjects from a non- endemic area were tested and used as a control. The subjects aged between 5 years to >30 years were used who has not been treated with schistosomiasis previously.

III. Methodology

A. Preparation of Lateral Flow Strip

The strip was constructed using sample pads, glass fibre conjugate pads, nitrocellulose membrane and cellulose absorbent pads and assembled onto a plastic adhesive backing card.). Conjugate pad and absorbent pad were placed at opposite ends of the nitrocellulose membrane, overlapping the membrane by approximately 2 mm to allow the flow of liquid through the strip by capillary action. Biotin (0.6 mg/ml-neat) was diluted at 1:8 and dispensed as a control line while anti-FITC (6.7 mg/ml-neat) was diluted at 1:16 and dispensed as a target line onto the nitrocellulose membrane. The strip was then and dried at room temperature in drying cabinet with a relative humidity (RH) around 20 - 30% for 24 hours

B. Preparation of Dried Streptavidin-Gold Conjugate

The amount of 225 µl of OD 20 streptavidin-gold conjugates were diluted into 675 µl of conjugate buffer to a final concentration of OD 5, dispensed onto a 15 cm of glass fibre conjugate pad and dried at room temperature in drying cabinet with a relative humidity (RH) around 20 to 30% for 24 hours before strip assembly. The assembled card was cut into 5 mm test strips following the standard operating procedure of the strip cutter.

C. Performing LATE-PCR LFA Strip from Amplified LATE-PCR Product

Onto the sample pad ten microliter of the LATE-PCR product was applied adjacent to the conjugate pad and 140 ul of standard LFA running buffer (10mM PBS of pH 7.4, 1% BSA and 0.05% Tween-20] was placed onto the sample pad (Faizul, 2014)..The mixture migrated towards the absorbent pad by capillary attraction. The result can be visualized in the form of red lines by using naked eyes within fifteen minutes.

D. Interpretation of LATE-PCR Dipstick LFA Strip Result

Presence of all two lines (control, and target) along the strip indicates valid positive results. Presence of only one line at control (i.e control band only) indicates a valid negative result. Illustration of LATE-PCR LFA Strip results are shown in Fig. 3 below.

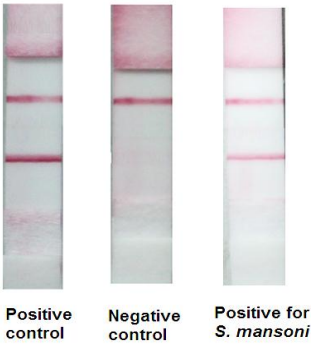

Figure 3. Showing positive control strip, negative control strip and Positive for target S.mansoni

IV. Results

Evaluation study on 50 stool samples showed 100% sensitivity and specificity suggesting the primers and designed probe is specific without any cross- reaction with other microorganisms. The sensitivity of LATE-PCR was said to be higher when compared to parasitological methods (Microscopy). LATE-PCR dipstick is highly sensitive since 20 positive stool samples with very low eggs burden were also seen to be positive by the test (Table I).

The capture reagents used in the development of S.mansoni LATE-PCR dipstick, which were biotinylated anti-mouse IgG and monoclonal anti-FITC(anti-FITC).The concentration of each capture reagent was optimized in order to obtained the optimum intensity of the red line on the LFA strip.

The optimization of the capture reagents was initiated using the control red line which is biotinylated anti-mouse IgG.

TABLE I. Evaluation Study on 50 Stool Samples Showed 100% Sensitivity and Specificity Suggesting the Primers and Designed Probe Is Specific without Any Cross- Detection with Other Microorganisms

Category	Late-PCR dipstick for the detection of *S. mansoni*		Total
	Positive	Negative	
Microscopy positive	20	0	[a] 30 (100%)
Microscopy negative	0	30	[b] 20 (100%)
	[c] 30 (100%)	[d] 20 (100%)	50

a: Sensitivity

b: Specificity

c: Positive predictive value (PPV)

d: Negative predictive value (NPV)

The biotinylated anti-mouse IgG was diluted serially in 10m M PBS (pH 7.4).One microliter (ul) of each diluted biotinylated anti-mouse IgG was dispensed into the nitrocellulose membrane and dried in drying cabinet for 24 hours with RH 20%, to 30%. An amount of 140ul of a running buffer was dispensed onto the sample pad and then allowed to migrate towards the absorbent pad for 15 minutes. The intensity of red line was then visualized.

Towards the absorbent pad for 15 minutes, the intensity of red line could then be visualized.

V. Discussion

Parasitological techniques which seems to be a gold standard for the detection of schistosoma eggs in stool which are often reliable in areas that are highly endemic for schistosomiasis. However, the number of false negative increases when the eggs intensity is low. In some rare situation when the eggs of *Schistosoma mansoni* in stool is low or fall below 100 eggs/gram (epg). It may become difficult to ascertain whether there is an infection or not [10]. Some of the limitations found with the parasitological techniques is that of time consuming, less cost effective, labour intensive as well as low sensitivity [11].

The result of our finding showed that Schistosomamansoni was found to be endemic in the study area of North-western, Nigeria. In an attempt to overcome short comings of the current diagnostic methods for detection of schistosomiasis mansoni, we have developed a laboratory tested LATE-PCR dipstick that may be suitable for this purpose. Since the LATE-PCR dipstick assay was based on detection and amplification of a highly repeated DNA sequence. LATE-PCR dipstick being highly sensitive than the conventional PCR and this is demonstrated by the detection limit of as low as 1ng/ul of S.mansoni in stool sample. With regards to specificity of the present testThe amplification reaction showed to be specific without any cross reaction with DNA from other intestinal micro-organism The LATE-PCR dipstick assay enable identification of *S. mansoni* as long as the DNA of the organisms remain intact in the sample to be tested.

In this study, the LATE-PCR dipstick parameters were optimized and detection of PCR products was performed on 2% agarose gel electrophoresis and the nitrocellulose membrane was coated with biotinylated anti-mouse IgG (control line), anti-FITC (target line) and assembled as lateral flow strips.The present Study showed that the developed LATE-PCR dipstick is a promising tool for the specific, sensitive and rapid diagnosis of infection with *schistosoma mansoni*. It is advice that future research studies on the application of this DNA based assay should be carried out with other schistosome specie and with the hope of evaluating its applicability in the field as well as in the follow up of chemotherapy.

VI. Conclusion

The present study findings revealed that LATE-PCR dipstick presented a high sensitivity and specificity in low

endemic areas and therefore could serve as a valuable alternative method for diagnosis of Schistosomiasis mansoniinfection in underdeveloped as well as in developing countries.

There is the needs to evaluate and applied this DNA based assay as a screening tool in areas of low endemicity because of the techniques being simple, rapid, sensitive and equipment free assay.

ACKNOWLEDGEMENT

The Authors would like to acknowledged the Support and assistance provided by institute of postgraduate studies, University Sains Malaysia (USM). We would like to acknowledge state ministry of health, Sokoto, Zamfara and Kebbi state for their approval to carry out this study. We also acknowledge Human ethics committee Universiti Sains Malaysia for their approval. We are grateful to parents/Guardians of the school children and adults household for their co-operations and willingness to participate in the study.

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