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Short technical report

Detection of schistosomes polymerase chain reaction amplified DNA by oligochromatographic dipstick

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ABSTRACT

The applications of highly specific and sensitive molecular techniques based on polymerase chain reaction (PCR) have constituted a valuable tool for the diagnosis of schistosomiasis and also for the detection of schistosome infections in the snail intermediate hosts. The common method of detecting PCR amplicons is gel electrophoresis in the presence of ethidium bromide, a carcinogen, which is followed by UV transillumination. Other methods, which are available for detecting PCR products, are real-time PCR, PCR–enzyme-linked immunosorbent assay (PCR–ELIZA) and mass spectrometry but they are cumbersome while they are sometimes complex and expensive. Therefore, a simple method of PCR product detection would be a welcome idea and a most valuable tool particularly in disease endemic countries with limited research facilities and resources. In this study, we applied a simple and rapid method for the detection of *Schistosoma haematobium* and *Schistosoma mansoni* PCR amplified DNA products using oligochromatographic (OC) dipstick. The amplicons are visualized by hybridization with a gold conjugated probe, while a control for the chromatographic migration is incorporated in the assay. The lower detection limit observed was 10 fg of genomic DNA from each of the two species, while the dipstick was also specific for each of the species used in this study.

1. Introduction

Schistosomiasis, a vector-borne parasitic infection is a serious, debilitating and sometimes fatal disease that afflicts about 200 million people living in developing countries [1]. It is caused by parasitic helminths of the genus *Schistosoma* and transmitted via freshwater snail intermediate hosts. It is more prevalent in the rural riverine areas and in areas where developments of projects to promote irrigation and provide hydroelectric power are taking place [2]. Their distributions are focal, aggregated and are usually related to the presence of several man-made impoundments, small seasonal streams, irrigation canals and ponds with numerous endemic foci all over the country [3].

Schistosomiasis control programs are chiefly based on treatment of infected populations, hence adequate case finding is crucial. Up to now, the available diagnostic methods of schistosomiasis are search for eggs in stools or urine and detecting eggs or adult worm antigens in urine and sera of infected individuals. Surveillance techniques include monitoring schistosome eggs output by humans, human water contact activities, snail infection rates and in some cases, numbers of cercariae in the water [4]. Detection of eggs or adult worm antigens in urine and sera of infected individuals can differentiate between past and current infections with specificity close to 100%, however, the disadvantages include low sensitivity in light infections, high cost, difficult approach and dependence on monoclonal antibodies and recently a polymerase chain reaction (PCR) was developed for the detection of schistosome DNA in faeces [5]. Available detection methods of schistosome infections in snails include snail crushing in search of larvae, repeated shedding of cercariae in the laboratory, detection of schistosomal antigens in snail hemolymph [6] and also polymerase chain reaction assays [7–15]. General method of detecting PCR products is gel electrophoresis in the presence of ethidium bromide, a carcinogen, followed by UV transillumination. Others are real-time PCR, PCR–enzyme-linked immunosorbent assay (ELIZA) and mass spectrometry. However, these methods are cumbersome, sometimes complex and expensive [16].

A simple method of PCR product detection would be a welcome idea and a most valuable tool, particularly in cases of low endemicity and parasitemia. Oligochromatographic (OC) dip-

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stick (Coris BioConcept, Gembloux, Belgium; patent number WO 2004/099438A1) is a simple and rapid method for detection of amplified PCR products [17–20], which are visualized by hybridization with a gold conjugated probe, while a control for the chromatographic migration is incorporated in the assay. A dry heating block or water bath and a micropipette are the only equipment needed for the detection protocol. In this study, we present the results observed using the OC dipstick to detect the PCR amplified gDNA from *Schistosoma haematobium* and *Schistosoma mansoni*. The OC dipstick was also used to detect PCR amplified gDNA from other schistosome sp. that could cross-hybridize with either *S. haematobium* or *S. mansoni*.

2. Materials and methods

2.1. Schistosomes

Genomic DNA was extracted from the adults of various schistosome species supplied by the Wolfson Wellcome Biomedical Laboratories, Zoology Department, Natural History Museum, London, UK. The schistosome species used were as follows; *S. haematobium* from Loum, Cameroon; *S. mansoni* from Belo Horizonte, Brazil; *S. bovis* from Iranga, Tanzania; *S. curassoni* from Dakar, Senegal; *S. intercalatum* from Edea, Cameroon; *S. rodhaini* from Rodhaini, Burundi; *S. mattheei* from Denwood, Zambia; *S. magrebowiei* from Lochinvar, Zambia and they have since been kept frozen in liquid nitrogen before use.

2.2. Schistosomes genomic DNA extraction

Genomic DNA extraction was done using CTAB extraction buffer containing 2-mercaptoethanol; hexadecyltrimethyl-ammonium bromide (CTAB) (solid); *tris*(hydroxymethyl) amino-methane; ethylenediaminetetra-acetic acid, disodium salt solution (EDTA); sodium chloride. Each adult worm was placed into a sterile 1.5 ml eppendorf tube, 500 μl of CTAB solution was added followed by 10 μl of proteinase K solution (20 mg/ml) and incubated at 55 °C for 1 h, with occasional gentle mixing. Genomic DNA was extracted from the CTAB buffer by adding an equal volume of chloroform/isoamyl alcohol (24:1) to each tube. The organic and aqueous layers were gently mixed for 5 min and spun at 13,000 rpm for 20 min. The upper aqueous layer was removed into another sterile eppendorf tube and an equal volume of 100% ethanol was added, mixed and was incubated at −20 °C overnight in order to enhance DNA precipitation. The solution was spun at 13,000 rpm for 20 min and the pellet was washed with 70% ethanol and was spun for another 20 min. The supernatant was removed and the pellet was dried at room temperature. When completely dry, the pellet was resuspended in 50 μl of water.

2.3. S. haematobium

2.3.1. Template and primers

The primers used were biotin-labelled forward 5′ GATCTCACCTATCAGACGAAAC 3′ and non-labelled reverse 5′ TCACAACGATACGACCAAC 3′ based on the 97 base pair (bp) sequence of *S. haematobium* shown below. GATCTCACCTATCAGACGAAACAAAGAAAATTTTAAAATTGTTGGTGGAAGTGCCTGTTTCGCAATATCTCCGGAATGGTTGGTCGTATCGTTGTGA (Gen Bank accession number DQ157698.1) [7]. The DraI repeat is also found in the genome of *S. bovis*, *S. magrebowiei*, *S. mattheei*, *S. curassoni*, and *S. intercalatum* [21].

2.3.2. Detection probe-gold bead and migration control probe

Detection probe (coupled to gold beads as described in the patent WO 2004/099438A1 (1)) sequence was 5′ AACAGGCACTTCCACCAACAAT 3′ and migration control probe was 5′ ATTGTTGGTGGAAGTGCCTGTT 3′. The design of the detection probe was done to reduce competition between (i) the probe and the secondary structure of the detected strand and (ii) the probe and the forward primer, which was used in high concentration (Fig. 1).

2.4. S. mansoni

2.4.1. Template and primers

The primers used were biotin-labelled forward 5′ CGTCATCACCTTAAACATGAAC 3′ and non-labelled reverse 5′ AATCACCAATGGCACAATCT 3′ based on the 165 base pair (bp) sequence of *S. mansoni* shown below.

CGTCATCACCTTAAACATGAACAACGAATAACTGAAGCTACAGTTGATAATATTGATTCCACTTGTTCAATATCTCCTAGTTGTTATCACAAACAAGGTAATAATAATAATAATAATAATAATAATAGACAATCAAAAGAATCACAGATTGTGCCATTGGTGATT (Gen Bank accession number DQ137434.1) [22].

2.4.2. Detection probe-gold bead and migration control probe

Detection probe (coupled to gold beads) sequence was 5′ GAACAAGTGGAATCAATATTATCAACTG 3′ and migration control probe 5′ CAGTTGATAATATTGATTCCACTTGTTC 3′. The design of the probe was a compromise to reduce competition between (i) the probe and the secondary structure of the detected strand and (ii) the probe and the forward primer, which was also used in high concentration

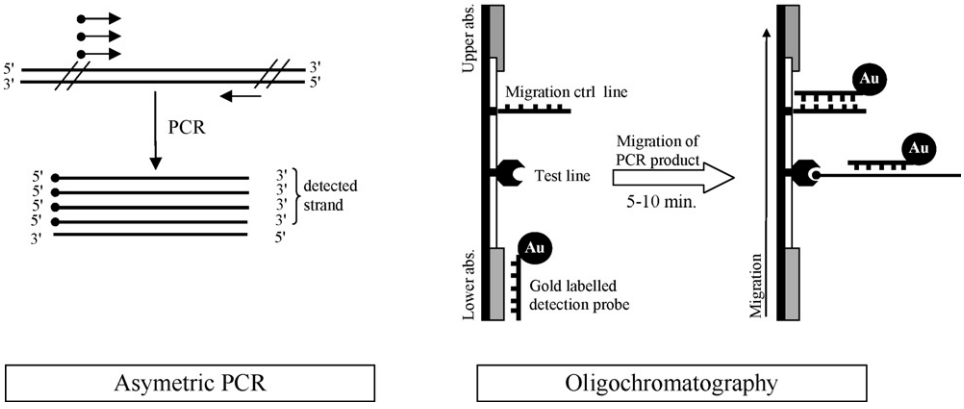

Fig. 1. Design of the PCR–oligochromatography assay.