Thesis Title Nonradioactive DNA Probe for Specific Detection of Human Malaria

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ABSTRACT

Malaria is still one of the deadliest and wide spread diseases in tropical countries including Thailand. Nonradioactive detection system is an alternative approach for detection of specific malaria parasite in blood samples other than microscopic examination. This technique is suitable for processing a number of samples simultaneously. A simple nonradioactive DNA hybridization assay for detection of Plasmodium falciparum in blood samples was developed. Among three commercial nonradioactive hybridization kits tested (the Genius™, the Photogene, and the Enhanced Chemiluminescent Detection System), the Genius™ System (digoxigenin method) was selected for optimization. The procedure involved spotting, five ul of blood collected in non-heparinized tubes onto GeneScreen Plus membrane. After drying for 1-6 hr, the dotted membrane was treated with 10 mM Tris 1 mM EDTA solution for 10 min, 0.5 N NaOH for 20 min, 1 M Tris-HCl pH 7.4 for 10 min, and 0.25% SDS solution for 30 min. These treatments were necessary for removing blood color background before further hybridization.

The probe was prepared from 1 ug of HindIII-digested Rep20 DNA (Rep20-H) using digoxigenin-dUTP labelling by random primed method. After 4 hr hybridization at 42°C, the probe was detected by using antidigoxigenin-alkaline phosphatase conjugate. Positive signals were shown up as blue precipitates on membrane or as dark spots on X-ray film when chromogenic or chemiluminescent substrates were added respectively. The processing time for detction of parasites was 12-14 hr. This modified digoxigenin method showed detection sensitivity of approximately 0.004% parasitemia determined by using parasites cultured in vitro.

In field application, 1316 blood samples collected from four different endemic areas in Thailand, i.e. Trad, Kanjanaburi, Mae Hongson, and Yala were examined by this modified digoxigenin method. One hundred blood samples (5 ul each) can be probed using with 1 ug of digoxigenin-Rep20-H DNA. The results revealed 11.7% disagreement when compared with clinic microscopy comprising 3 % false positives and 8.7% false negatives. However, the digoxigenin method had a reliable sensitivity of approximately greater than 0.01% parasitemia (25,000 parasites) when used to detect parasites in blood samples.