Use of Loop-Mediated Isothermal Amplification (LAMP) of DNA in Diagnosis and Monitoring Treatment of *Trypanosoma brucei rhodesiense* Infections in Vervet Monkeys (*Chlorocebus aethiops*)

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ABSTRACT

The recently developed loop-mediated isothermal (LAMP) based on *RIME* gene was used in diagnosis, staging and post-treatment follow-up of HAT in infected vervet monkeys (*Chlorocebus aethiops*) and compared with microscopic methods. The monkeys (A and B) were infected with *Trypanosoma brucei rhodesiense* 2537 and sub-curatively treated with diminazene aceturate (Berenil®) at 35 day of post infection (dpi) and later with Melarsoprol (Mel B®) at 113 dpi (78 day of post treatment (dpt) with Berenil®) and 171 dpi (136 dpt with Berenil®) respectively.

Pure and crude DNA from the samples for LAMP was extracted using Saponin method and heat treatment respectively. Amplification was conducted in a thermocycler and a water bath set between 60^0C to 65^0C. The test results were assessed visually by addition of SYBR green I dye and by ultra violet (UV) illumination of DNA bands in 1% ethidium bromide stained electrophoresed agarose gel. Parasitaemia, cerebrospinal fluid (CSF) parasitosis, packed cell volume (PCV), white blood cell (WBC) counts and total CSF protein concentration were also determined.

In the blood and CSF, microscopy detected 28.21% and 21.18% positive cases in the collected samples respectively. In the blood, serum and CSF, LAMP detected 60.26%, 55.13% and 79.49% positive cases in the collected samples respectively. The Chi-square (X^2)-Statistics of 16.734 (p=0.000 and 38.023 (p=0.000) was obtained between LAMP and microscopy in the blood and CSF, respectively.

The percentage trypanosome DNA detection on different sample preparation and amplification methods on LAMP was also assessed. Pure DNA on a thermocycler had 60.27%, 55.13% and 78.12% in the whole blood, serum and CSF respectively. For pure DNA on a water bath, the

percentage detection was 46.15%, 48.72% and 75.64% in the whole blood, serum and CSF, respectively. For crude DNA on a thermocycler, the percentage detection was 56.41%, 56.41% and 76.92% while crude DNA in a water bath had 48.72%, 44.87% and 64.10% detection rate was in the whole blood, serum and CSF, respectively.

Trypanosome DNA was detected at 7 dpi in the blood and serum and starting at 21 dpi in the CSF. After subcurative Berenil® treatment, trypanosome DNA cleared at 56 dpi (21 dpt with Berenil®) in the blood and serum of both monkeys, and re-appeared at 77 dpi (42 dpt with Berenil®) and 129 dpi (84 dpt with Berenil®) in the blood and serum of monkey A and B respectively. After Mel B® treatment, trypanosome DNA cleared after 40 and 90 days and 90 and 150 days in the blood and serum and CSF of vervet monkey A and B respectively.

The comparison between LAMP and microscopy for crude DNA on thermocycler had k values of; 0.397 and 0.602 and X^2-value of 13.141 (p=0.000) and 35.247 (p=0000) in the blood and CSF respectively. Percentage agreement (k) and X^2) between LAMP and microscopy in detection of trypanosomes in the late stages of the disease was 0.600 and 15.000 (p=0.000) respectively in the CSF.

For post-treatment follow-up the k and X^2 values were 0.472 and 19.429 (p=0.000) and 0.527 and 21.346 (p=0.000) in the blood and CSF respectively. Therefore, heat treatment and amplification on a water bath may be use as sample preparation and amplification methods respectively. Blood and CSF may are the preferred sample for early and late stage of the disease.