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Molecular techniques in more accurate and rapid detection of *T. brucei rhodesiense* in non-endemic countries

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We use this protocol and it's working



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Abstract

This protocol describes the methods for rapid detection of *T. brucei rhodesiense*, related to the manuscript Molecular techniques in more accurate and rapid detection of *T. brucei rhodesiense* in non-endemic countries.

Metagenomic analysis with Oxford Nanopore sequencing

- 1 Samples were extracted on an NucliSENS easyMAG extraction system (BioMerieux, Marcy-l'Étoile, France) with 200µl input and elution in 50µl. Sequencing libraries were prepared with the Rapid PCR Barcoding kit (SQK-RPB004, Oxford Nanopore Technologies, Oxford, UK) according to official protocol with modifications described here. Nine microliters of extracted DNA were mixed with 1µl of FRM and incubated at 30°C for 1 minute and 80°C for 1 minute and placed on a cooling block. To reduce amplification time of the PCR, the NextGenPCR system was used. Two microliters of tagged DNA, 10µl Arctic Fox HF Chemistry-2x (MBS, Goes, the Netherlands), 7,5µl nuclease-free water and 0,5µl RLB-barcode for each sample were prepared in duplicates and pipetted to an EZtrieve microplate (MBS, Goes, the Netherlands). The plate was sealed in the NextGenPCR Semi-automatic Heatsealer (MBS, Goes, the Netherlands) before being placed in a NextGenPCR thermocycler (MBS, Goes, the Netherlands). The PCR-program consisted of initial denaturation at 98°C for 1 minute followed by 5 cycles with denaturation at 98°C for 15 seconds, annealing at 56°C for 20 seconds and extension at 65°C for 2 minutes before 25 additional PCR cycles with denaturation at 98°C for 10 seconds, annealing at 56°C for 15 seconds and extension at 65°C for 1 minute. The duplicates were pooled, and the later steps were conducted according to protocol and libraries were loaded on a R9.4.1 flow cell with 1090 pores available (Oxford Nanopore Technologies, Oxford, UK) and sequenced for 72 hours on a GridION machine with MinkNOW version 22.10.5 (Oxford Nanopore Technologies, Oxford, UK) with Super accuracy basecalling applied during sequencing. Reads below a phred score of 10 and below 1 kilobase or longer than 15 kilobases were excluded by seqkit version 0.13.2 (1). Reads were mapped to the hg38 human reference genome (2) with minimap2 version 2.24 (3) and non-human reads were extracted with samtools version 1.16 (4) and analysed with Kraken2 version 2.12 (5). The non-human reads were mapped to the reference genome TREU927 *Trypanosoma brucei* (6) with minimap2. The coverage per position was extracted with samtools and median coverage across the major chromosomes was calculated with R version 4.3.0 (7).

A total of 3.4 M reads were generated after 72 hours with a median length of 3.5 kb.

ITS1 and SRA PCR amplification and Sanger sequencing

- 2 The extracted DNA from metagenomic analysis was also used as input for the specific ITS and SRA PCR-reactions. PCRs were performed using published primers from (7,8). Both of the PCRs were performed with 4µl DNA input template, 10µl Invitrogen 2X SuperFi II PCR master mix (Thermo Fisher Scientific, Waltham, MA, USA) 4µl of nuclease-free water, 1µl forward, 1µl reverse primer of either SRA or ITS primer sets. PCR were performed on an Applied Biosystems VeritiPro thermal cycler (Thermo Fisher

Scientific, Waltham, MA, USA) with initial denaturation at 98°C for 30 seconds followed by 35 cycles of denaturation at 98°C for 30 seconds, annealing at 60°C for 20 seconds and 98°C extension at 72°C for 1 minute before final extension at 72°C for 5 minutes. Eight microliter of amplicon and 12 µl of water were mixed and loaded on a 1.2% E-gel (Thermo Fisher Scientific, Waltham, MA, USA) together with the 1kb plus DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA). The samples were run 20 minutes and visually inspected for DNA bands of the correct size. Amplicons were diluted 1:10 and mixed with either of the forward or reverse primers and shipped for Sanger sequencing overnight with the Mix2Seq kit (Eurofins Genomics, Germany). Sequences were uploaded to the online blastn BLAST resource with standard settings (9).

DTA blood and anaerobic blood cultures were both PCR positive for ITS1 and SRA with gel bands between 400–500 bps and 200–300 bps, respectively. BLAST analysis of the SRA Sanger sequences confirmed the finding of *T. b. rhodesiense*.

Protocol references

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