2 OBJECTIVES

The specific objectives and planned activities of the study were as follows:

1. To assess the isometamidium sensitivities of *T. congolense* clones derived from trypanosome stocks collected from naturally infected cattle.
   Activities: Generate clones from *T. congolense* field stocks from Ethiopia and Burkina Faso and characterise them for their isometamidium sensitivity using the standard protocol described in Eisler et al. (2001).

2. To induce isometamidium resistance in a drug sensitive *T. congolense* clone in immunosuppressed mice, with the aim of understanding the developmental mechanism of resistance in trypanosomes.
   Activities: Derive *T. congolense* clones with different level of resistance to isometamidium from a drug sensitive clone in immunosuppressed mice and test the stability of the drug resistance phenotype in immunocompetent mice against isometamidium and diminazene.

3. To characterise the putatively resistance-related nucleoside transporter gene (TbAT1) in isometamidium sensitive and resistant *T. brucei brucei* field and reference stocks.
   Activities: a) PCR analysis of the *T. brucei* study stocks using nuclear repeat primers (Moser et al., 1989) specific for *Trypanozoon* and primers complementary to the SRA gene fragment (Radwanska et al., 2002); b) Genomic DNA amplification of the TbAT1 gene fragments (Mäser et al., 1999) in *T. b. brucei*; c) Analyses of sequence variations in TbAT1 gene-fragments between phenotypically characterised isometamidium sensitive and resistant *T. brucei brucei*, and analyse if there is a link between mutations in this gene and isometamidium resistance; d) RFLP analysis of a fragment of TbAT1 gene from the isometamidium sensitive and resistant *T. b. brucei*.

4. To identify putatively resistance-related sequences in *T. congolense* homologues to the nucleoside transporter gene sequences (TbAT1) described in *T. brucei*.
   Activities: a) PCR analysis of the *T. congolense* field isolates with different drug sensitivity phenotypes from Ethiopia and Burkina Faso to determine whether they are savannah or forest types; b) PCR analysis of genomic DNA of different *T. congolense*

clones for the presence of purine transporter genes described in *T. brucei*; c) PCR analysis of genomic DNA samples from different *T. congolens*e study clones using degenerate primers designed from TbAT1 gene sequences.