

THE POTENTIAL USE OF RECOMBINANT PROTEIN IN ENZYME-LINKED IMMUNOSORBENT ASSAY AND IMMUNOCHROMATOGRAPHIC TEST IN THE DIAGNOSIS OF AFRICAN TRYPANOSOMOSIS

Kennedy Miyoro Mochabo^{1, 2}, Thu-Thuy Nguyen¹, Keisuke Suganuma¹, Mo Zhou¹, Shin-ichiro Kawazu¹, Yasuhiko Suzuki³ and Noboru Inoue¹

¹National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, JAPAN

²Kenya Agricultural Research Institute, Trypanosomiasis Research Centre (KARI-TRC) Kikuyu, KENYA

³Research Center for Zoonosis Control, Hokkaido University, JAPAN



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Introduction

African Trypanosomosis

Animal

- *Trypanosoma brucei*
- *T. congolense*
- *T. vivax*

Human

- *T. brucei rhodesiense*
- *T. b. gambiense*

Tsetse fly

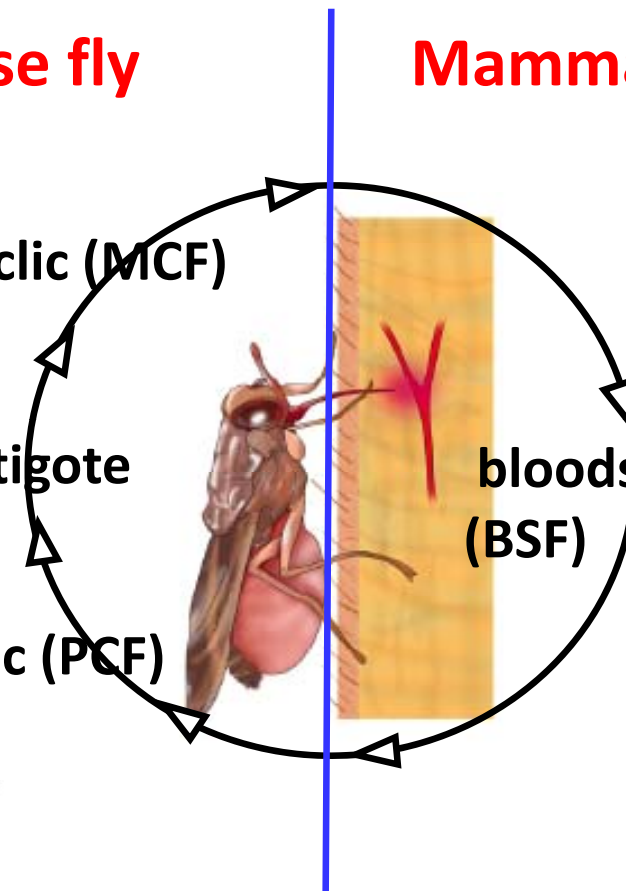
Mammal

metacyclic (MCF)

epimastigote (EMF)

procyclic (PCF)

bloodstream (BSF)



Differential stage-specific average fold of expression change

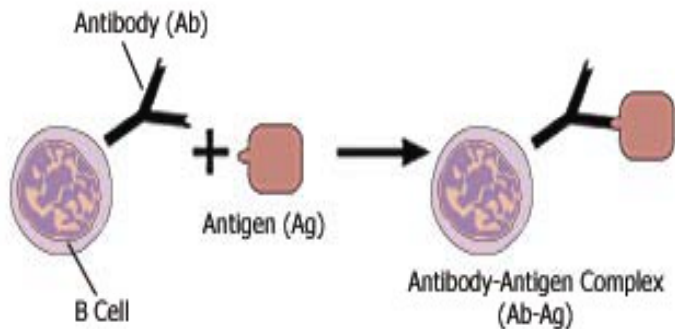
Accession #	Length bp	PCF/BSF	EMF/PCF	MCF/EMF	BSF/MCF
TcIL3000.0.38630	1236	0.15	1.74	8.47	0.62

Eyford et al., 2011

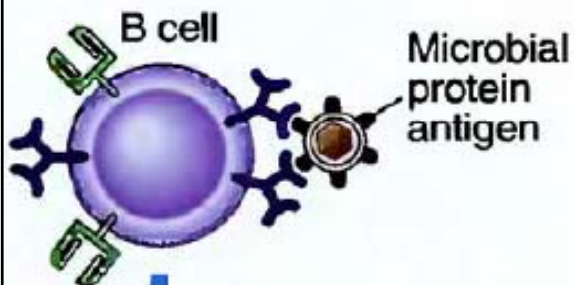
Orthologue of the *T. brucei* invariant surface glycoprotein (ISG)

There is a limitation in the diagnosis and treatment of the disease

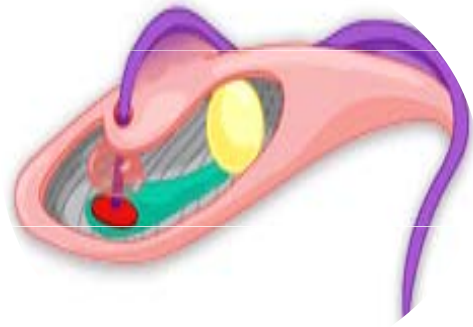
Introduction...




- Serological tests have been used for diagnosis of diseases – ELISA and ICT.
- ELISA was used in the early 70s – is suitable for mass screening.
- ICT was first used in home-based diagnosis for blood glucose screening and pregnancy testing immunoassays to be sold over the counter in the mid 1970s.



- Nowadays ICT has been adopted for detection of pathogens, both in animals and humans.



 The aim of this study was to find a new ELISA-based diagnostic test using recombinant proteins for the diagnosis of *T. congolense* infections and also apply it in ICT



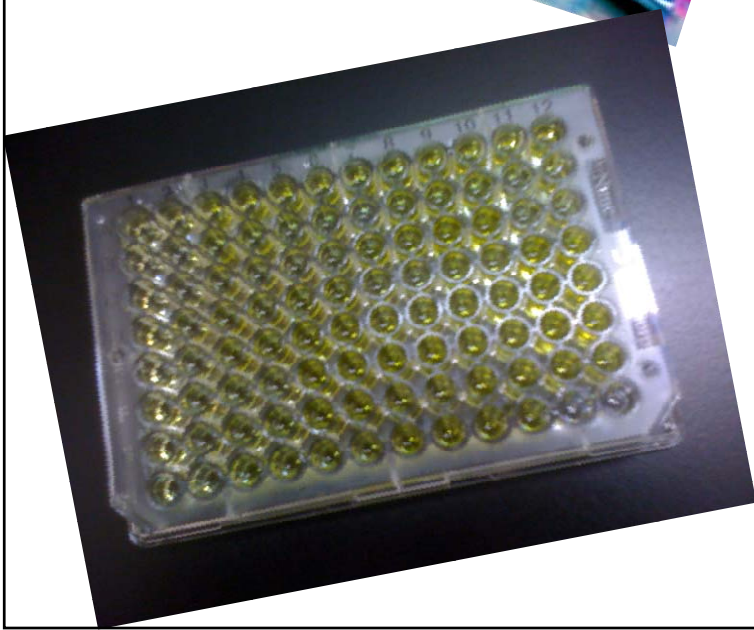
- ❑ Four mice were infected IP using *in vitro* BSF *T. congolense*
- ❑ Sera were collected on alternate days as day 0 (Pre-infection), 2, 4, 7, 9 ... for the first one month.
- ❑ Thereafter, weekly until the experiment was terminated at 90 days.



Materials and Methods...



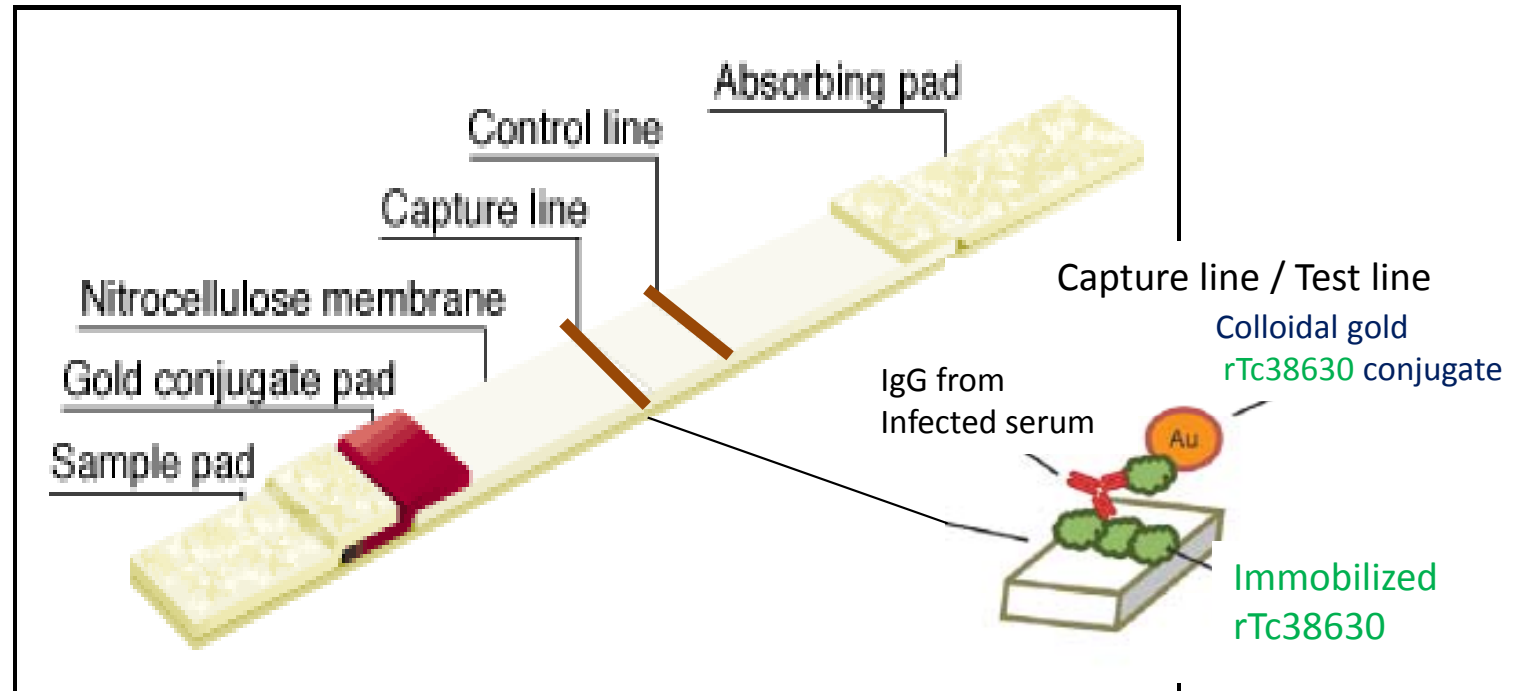
- Recombinant Protein with GST tag was purified and used as antigen in ELISA and ICT
- ELISA was performed on serially collected infected mice serum



Coating ag 200 ng/well
x200 dilution of sera
TMB substrate; 450 nm

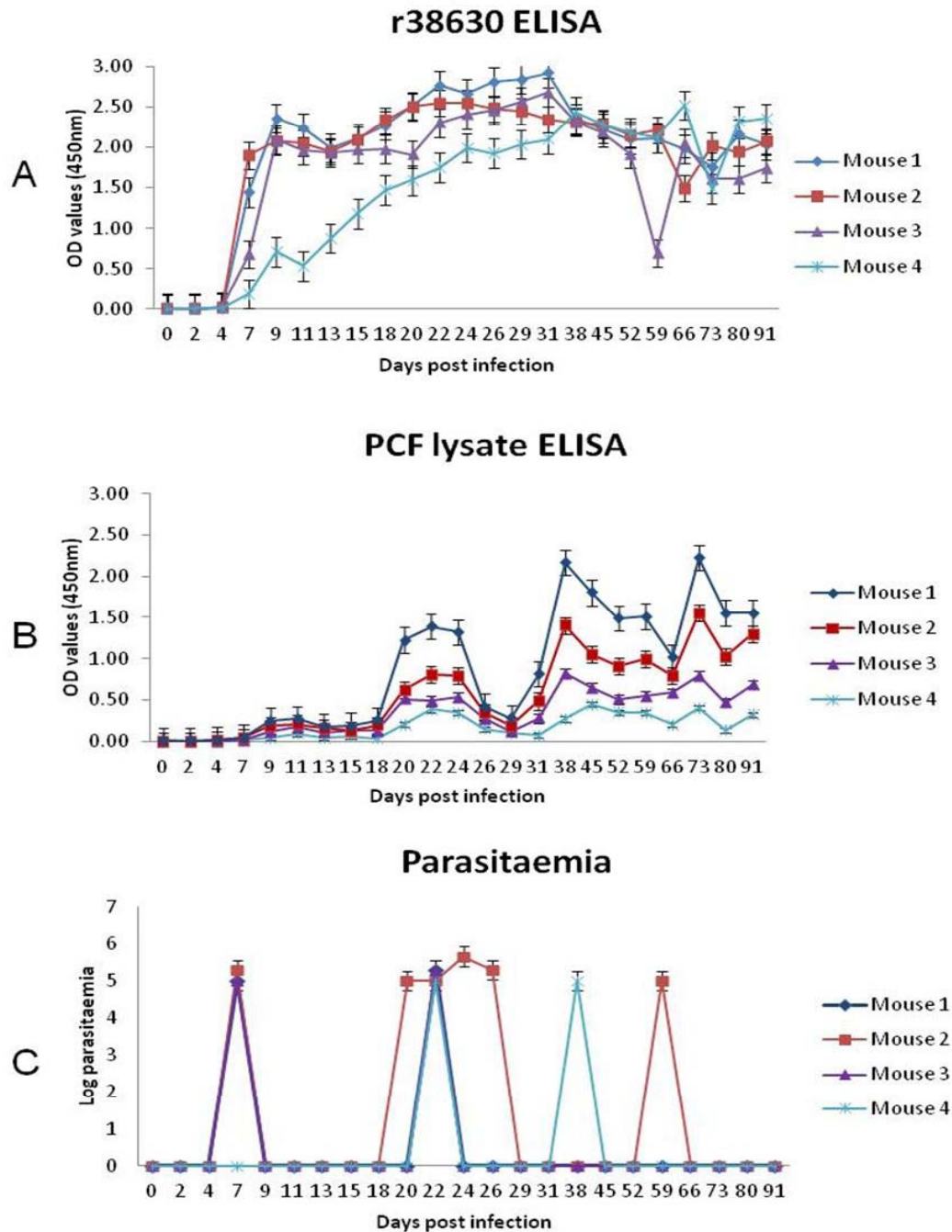
Materials and methods...

A typical immunochromatographic test strip



- Control line, GST line (Liao, 2005) and Capture line were jetted 5 mm apart on NC Membrane
- The parts were assembled in a continuous manner for capillary effect
- ICT card was cut into 3 mm wide strips using a cutter
- Conjugate pad = Colloidal Gold + Recombinant antigen (rTc38630)
- Testline/ Capture line = rTc38630
- Control line = Mouse polyclonal IgG (against rTc38630)

ELISA RESULTS ON MOUSE SERA



- Detection of IgG responses against rTc38630 (A) and PCF cell lysate (B) antigens in infected mouse sera by ELISA. Course of parasitaemia in infected mice is shown in panel C.
- Cut-off value 0.013 (mean + 3 standard deviation for day 0 sera).

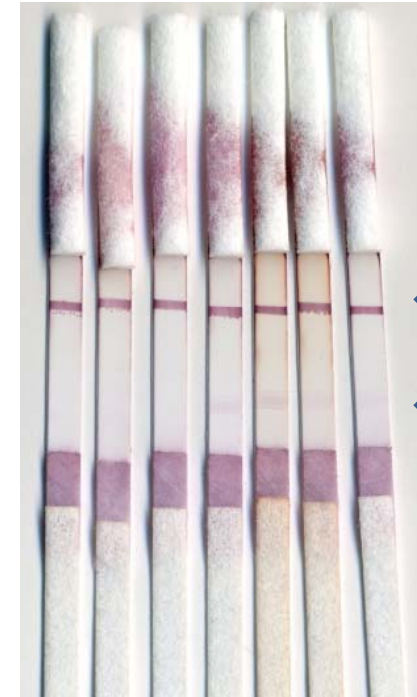
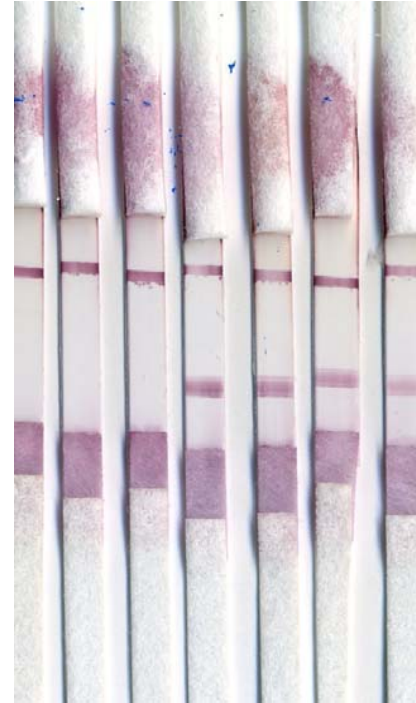
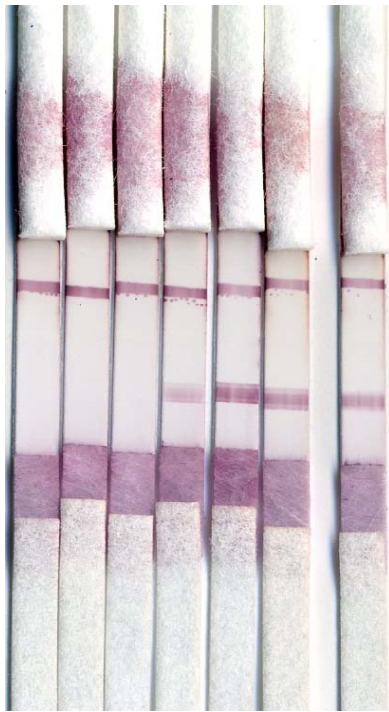
ICT Results on Mouse Sera

Mouse 1

Mouse 2

Mouse 3

Mouse 4



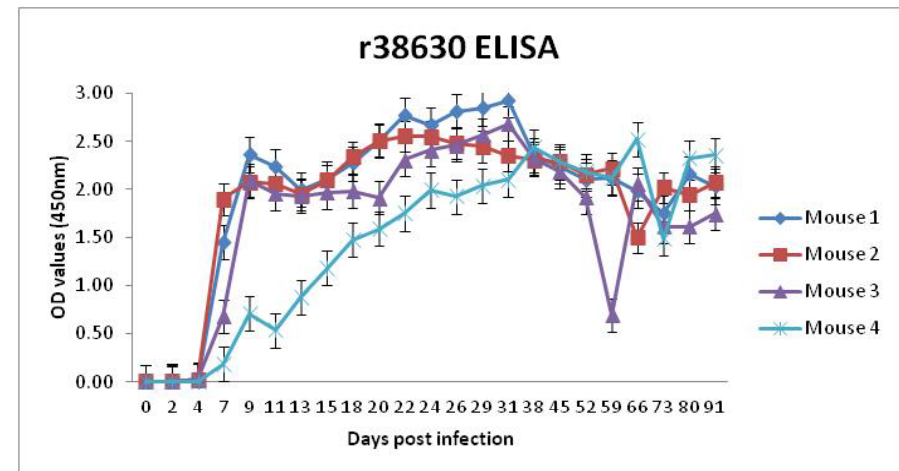
0 2 4 7 9 11 13

0 2 4 7 9 11 13

0 2 4 7 9 11 13

0 2 4 7 9 11 13

0-13 = Days Post Infection
Control line = Blue arrowed
Test line = Red arrowed



 **The result was consistent with recombinant protein ELISA**



Conclusions

- Overall, rTc38630 ELISA & PCF cell lysate ELISA were statistically not different ($p > 0.05$).
- From day 7 the rTc38630 ELISA was better in diagnosis of infection compared to day 9 by PCF lysate ELISA ($p < 0.05$) suggesting that rTc38630 protein would be used for early detection of trypanosomes.
- The ICT was consistent with the ELISA on infected murine model sera.
- The next plan is to apply rTc38630 protein in ICT and ELISA on field samples.

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