

Genetic mapping of *Trypanosoma congolense* populations

Y A Helmy, J Kruecken, P-H Clausen, V Delespaux, T Marcotty,
L Morrison, A MacLeod



Co-operating Partners



Sample acquisition



Technical support



Yosra Ahmed Helmy
(PhD student, DAAD-sponsored)



Establish an effective test protocol detecting genetic exchange (WP 3)

Screen blood samples from study sites in Togo, Ethiopia and Mozambique, exploring drug resistance dynamics (WP3)

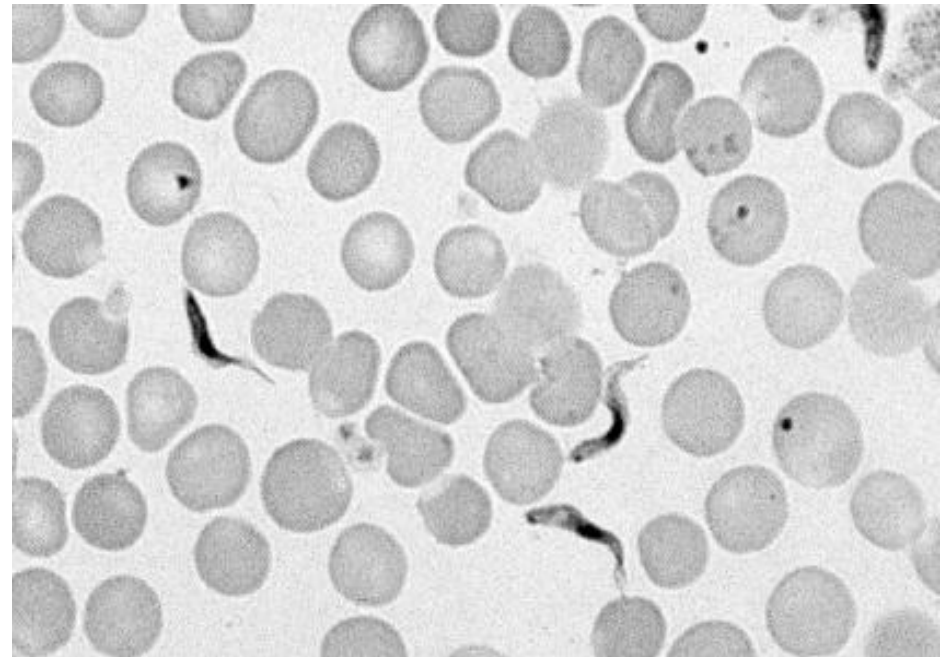
Identify and promote adapted strategies to improve the effectiveness of trypanocides thereby minimizing and controlling risk of trypanocide resistance (WP4)

Source: EuropeAid/128500/A/ACT/Multi – Annex 1 – Description of Action



Preliminary analysis of pre- and post-treatment populations of *T. congolense* in Mali

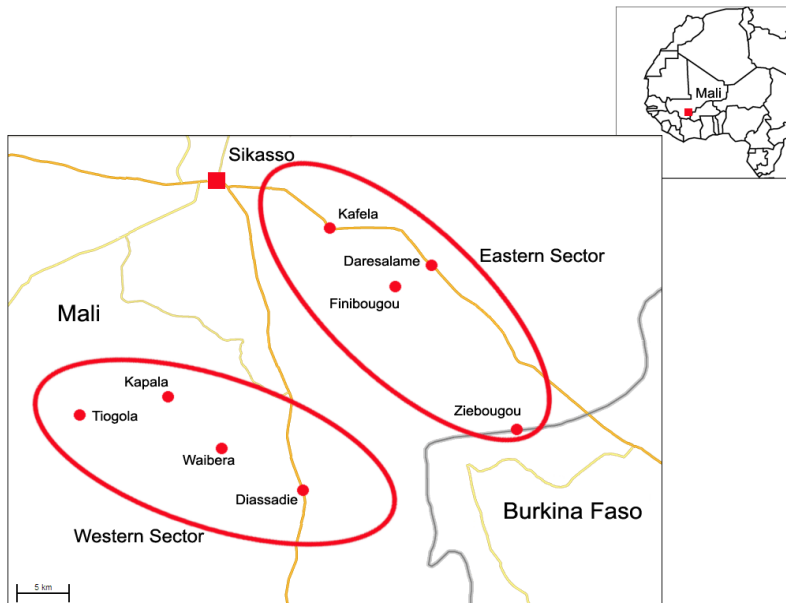
L Morrison (preliminary report, 2012)



Where the samples come from

Management of trypanocidal drug resistance in cattle in identified chemo resistance hot spots in the administrative District of Sikasso, south-east Mali (2007-2010)

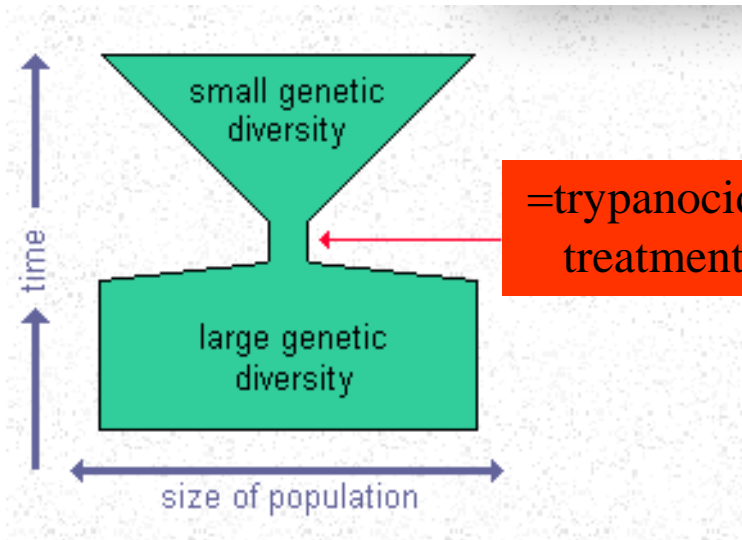
Erick Mungube Ouma, 2010, 1-197, Thesis, Freie Universität Berlin, Journal No. 3419



Material & Methods

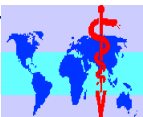
1. **DNA extraction** of blood samples on FTA cards
2. **Detecting T.c.-positive samples** by TCS-primers (Masiga *et. al.*, 1992)
3. **Detecting genetic variability** of highly repetitive non coding T.c.- DNA regions (=microsatellites) by TCM-primers (Morrison *et. al.*, 2009)

Principle:



→ resistance

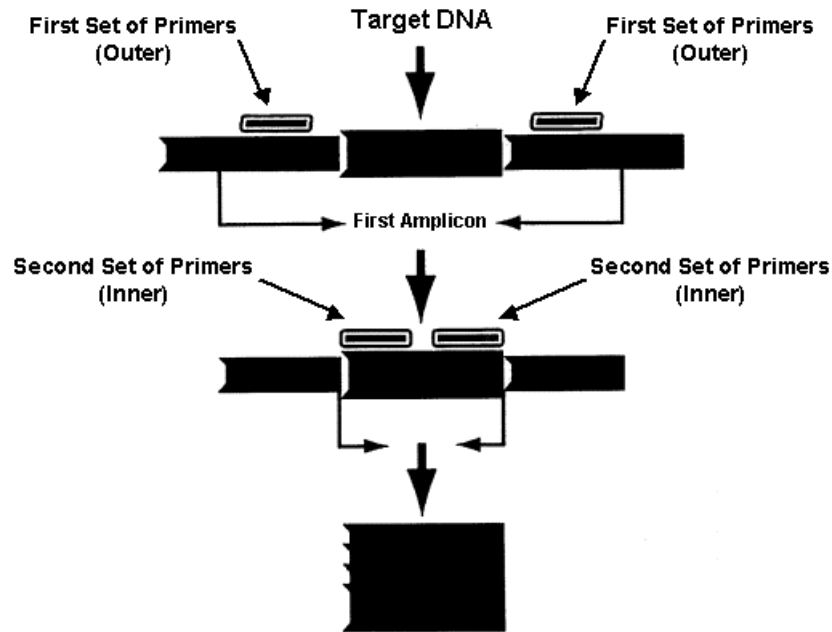
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Material & Methods

Problems:

- ➔ Very few positive pre- and post-treatment samples (2)
- ➔ Low numbers of template DNA per sample: solution **Nested PCR**



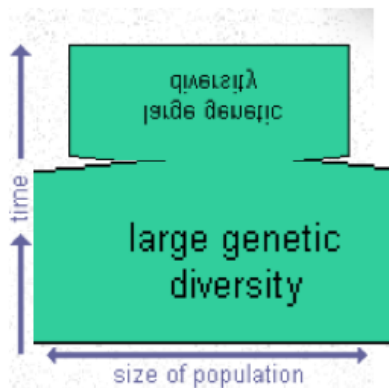
Specific Amplification of the Target DNA



Results

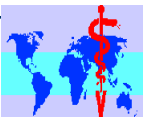
Sector	Response 14 days post-treatment with					
	DIM (n=62)			ISM (n=63)		
	No.	Relapsed	Failure rate (%)	No.	Relapsed	Failure rate (%)
<u>Eastern sector</u>						
<i>T. c</i>	21	10	47.6	20	12	60
<i>T. v</i>	6	0	0	8	0	0
Total	27	10	37.0	28	12	42.9

Source: Erick Mungube Ouma, 2010, 1-197, Thesis, Freie Universität Berlin, Journal No. 3419

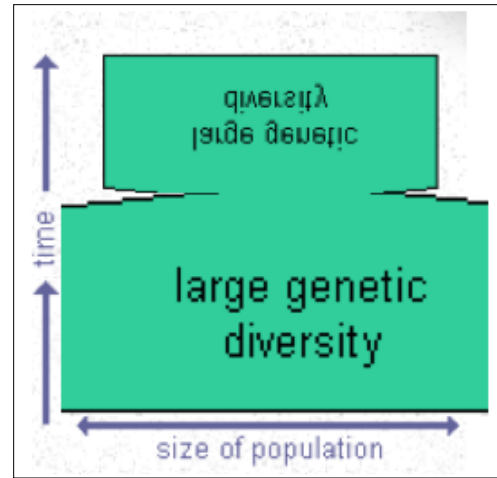


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➔ No changes in diversity
No severe bottleneck



Interpretation



Theory 1

Treatment failure

Theory 2

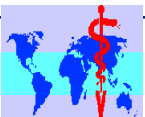
Initially low genetic diversity
due to longterm drug
mismanagement

Solution: Investigate more samples





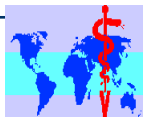
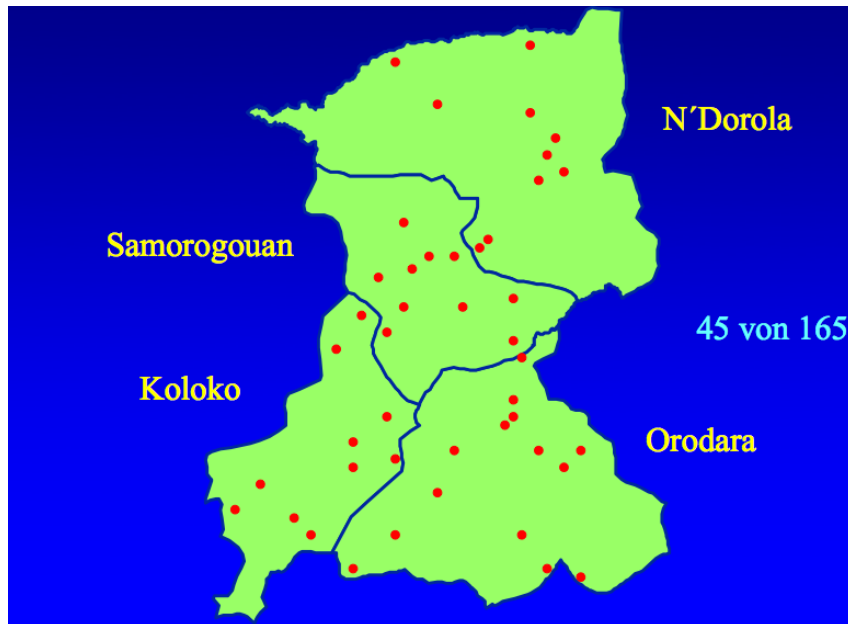
Genetic variability in *Trypanosoma congolense* from Kéné Dougou (Burkina Faso) by microsatellite markers



Where the samples come from

Field studies of drug-resistant cattle trypanosomes in Kéné Dougou Province, Burkina Faso

McDermott J, Woitag T, Sidibé I, Bauer B, Diarra B, Ouédraogo D, Kamuanga M, Peregrine A, Eisler M, Zessin KH, Mehlitz D, Clausen PH. *Acta Trop.* 2003 Apr;86(1):93-103.



Objectives

Optimize the TCM PCR protocol in Glasgow

Establish the system at the FU Berlin lab

Analyse study site samples from Togo, Ethiopia and Mozambique

Detect resistance/ resistance mechanisms

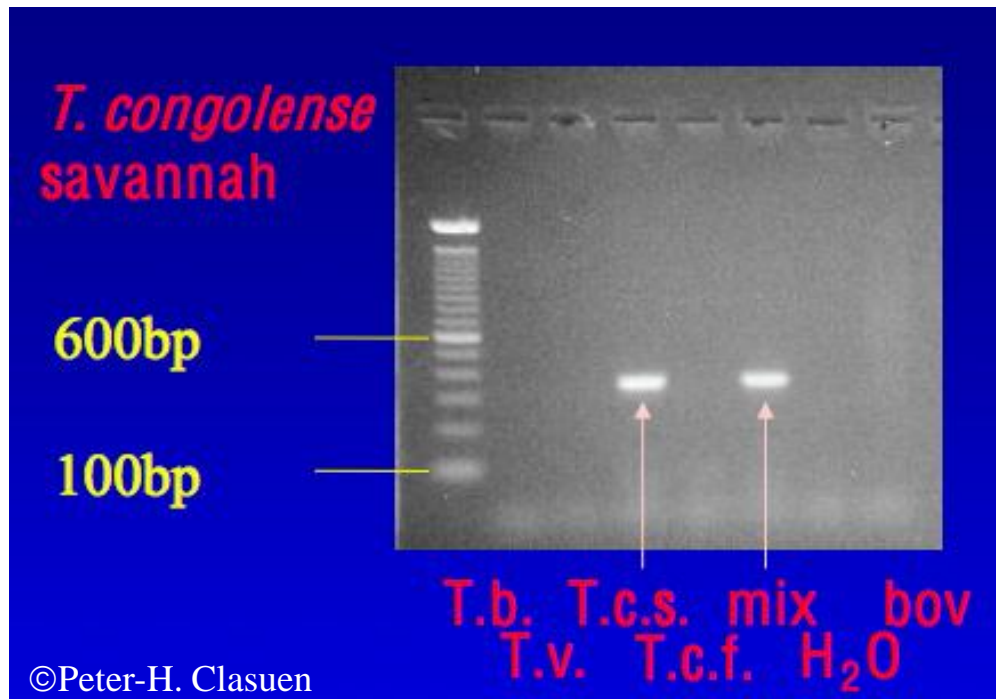


Material & Methods

180 DNA samples from infected cattle

Detecting *T.c.*-positive samples by TCS-primers

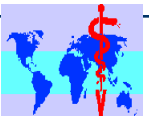
 → 36 samples



Material & Methods

Amplification with TCM (microsatellite) primers

Sequence analysis and interpretation



Preliminary Results: Optimizing the TCM primer protocol

Seven first round primer pairs

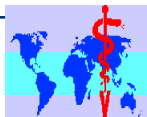
TCM1A TACAAATGACTGTAGAGCGGC
TCM1B CTGTGTGTATAATGATTCATTCG
TCM2A GGTAAGACAAAGTTGTGGGTG
TCM2B ATGTGACCGATGCTCCGAAC
TCM3A TCTATTGTTACAGTCTCGTG
TCM3B ACTCATTGCATAAAGGCTAG
TCM4A CTTAACGCTGCTTCAGTAGC
TCM4B AGTACACACGACTTCACCTCC
TCM5A CAATGGTTCAATAAGCGCACC
TCM5B AAGGCAAGTAAGTTACGC
TCM6A GAATGCGAGACCTGCTTCTTGG
TCM6B CATTAGACTCTCACTTTCCG
TCM7A GTGTAGTTTGTTATACTTCG
TCM7B GTTAAATACTTGTGAGAGCCAGC

and seven second round primer pairs

TCM1C CTAGAAGCGAGTAACAGCC
TCM1D AAGGGTTCGTACCACAGCCC
TCM2C CAGTCATGTATATGTTTGTG
TCM2D CCTGAAATGGGTCTACTGAG
TCM3C CATGCTCTTAGGTTCCATCGG
TCM3D AGCATCCGACATTGAAACGAC
TCM4C GTCTCTTCCGCACAGTGAC
TCM4D GGGGGAAGATATTAAGACAC
TCM5C CTTCCACGAGTCCCTAATCGACC
TCM5D TTGCTCACTGTCAAGGCGTGC
TCM6C AACCACCACTTCCGTGCACCGG
TCM6D CCATGAGCTTTATGCGACCTCTAC
TCM7C TCATAGAGGCAAGTGCGTAGC
TCM7D CCAGAATAAGAATACTTACTGC

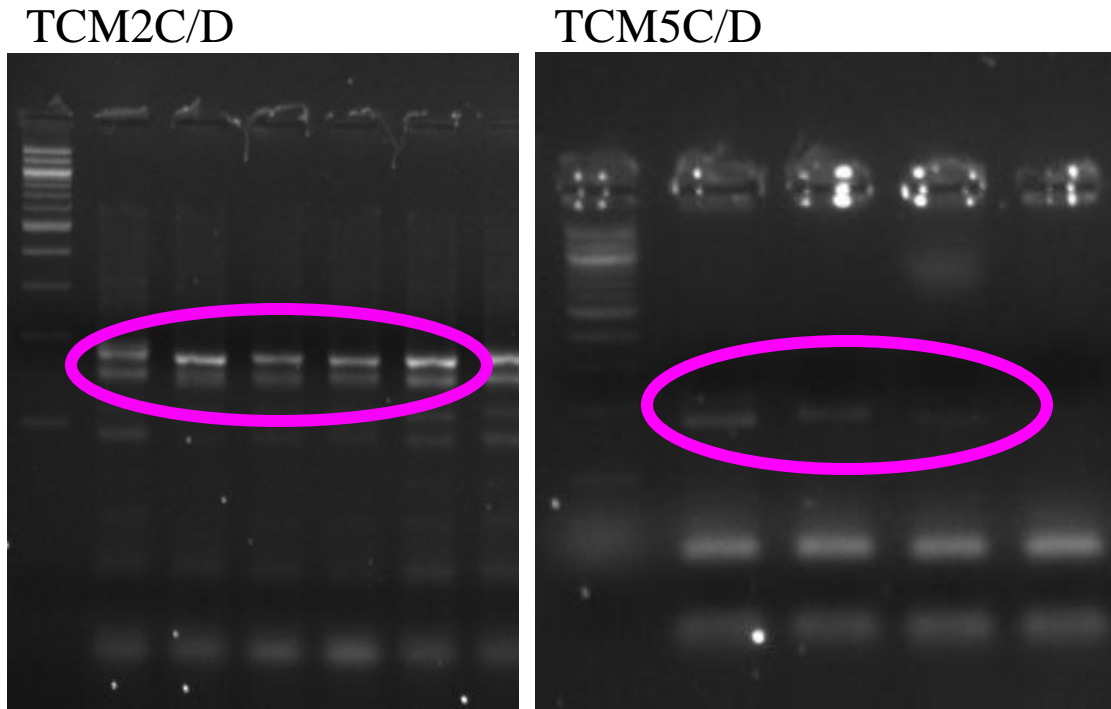
PCR runs with entirely second round primers now

Except for: TCM 2 not working yet → currently switching to hotstart polymerase



Preliminary Results:

Amplification with TCM 1-7 (microsatellite) primers 



→ fluorescence-marked products to be sent to genescan

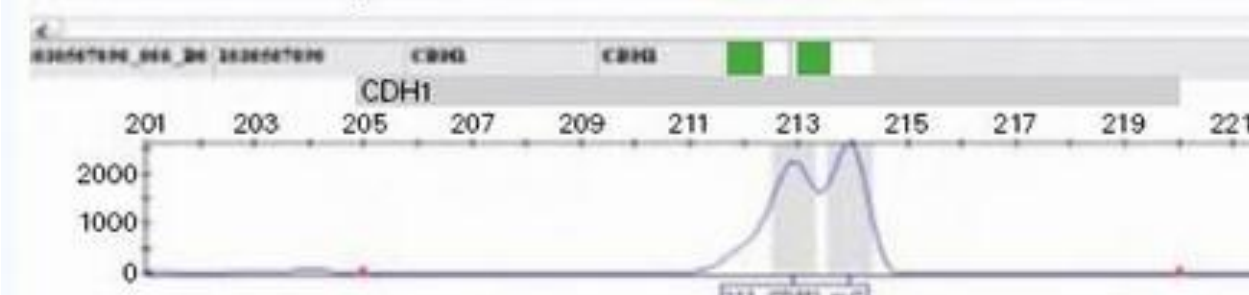
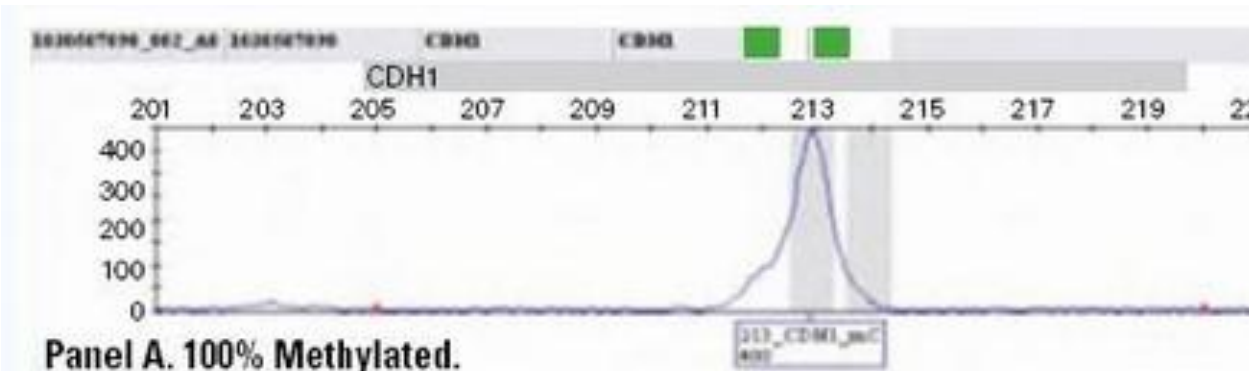


Microsatellite sequence of DNA regions

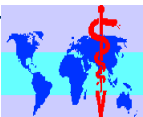


Bioinformatic data analysis by Peakscanner™ Software

One allele in a genescan peak image



<http://www.genoproof.de/de/qualitytype/rohdatenauswertung>



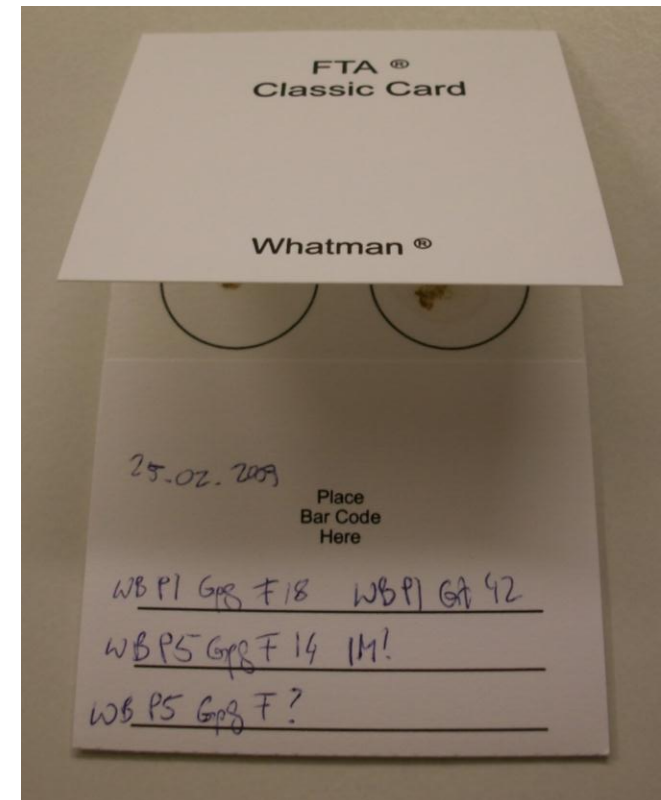
Increase DNA yield

Sample Storage: FTA[®] cards

+ Inactivate pathogens, storage up to 6 mo.'s

- Reported difficulties when parasitaemia is low

Cox AP, Tosas O, Tilley A, Picozzi K, Coleman P, Hide G, Welburn SC. Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. Parasit Vectors. 2010;3:82. doi: 10.1186/1756-3305-3-82.



DNA yield of FTA cards with various extractions:

Sensitivity for *T. brucei* DNA on FTA[®] cards (Ahmed *et. al.*, 2011):

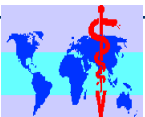
Chelex [®] 100 extraction from FTA [®] cards (10x 0.2x0.2 mm discs)	56.4% → 10x ?
DNA extraction from whole blood expensive?	68.3% → too
Lysed blood on FTA [®] cards	73.3% → too tedious?

→ Concentrate parasites in the blood tube through centrifugation

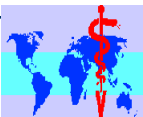
→ Spotting the buffy coat onto the FTA[®] card

and/or

→ Use the entire FTA[®] sample (about 3x3 cm/ 150 µl) for Chelex[®] 100 resin extraction



- Further colaboration
- Continue optimizing the protocol (FTA or whole blood?)
- Transfer the complete protocol to Berlin
- Analysis of field samples
- Apply knowledge in the field, publish





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NEVER BE AS COOL AS DARTH
VADER IN A KILT, ON A UNICYCLE,
PLAYING BAGPIPES!**