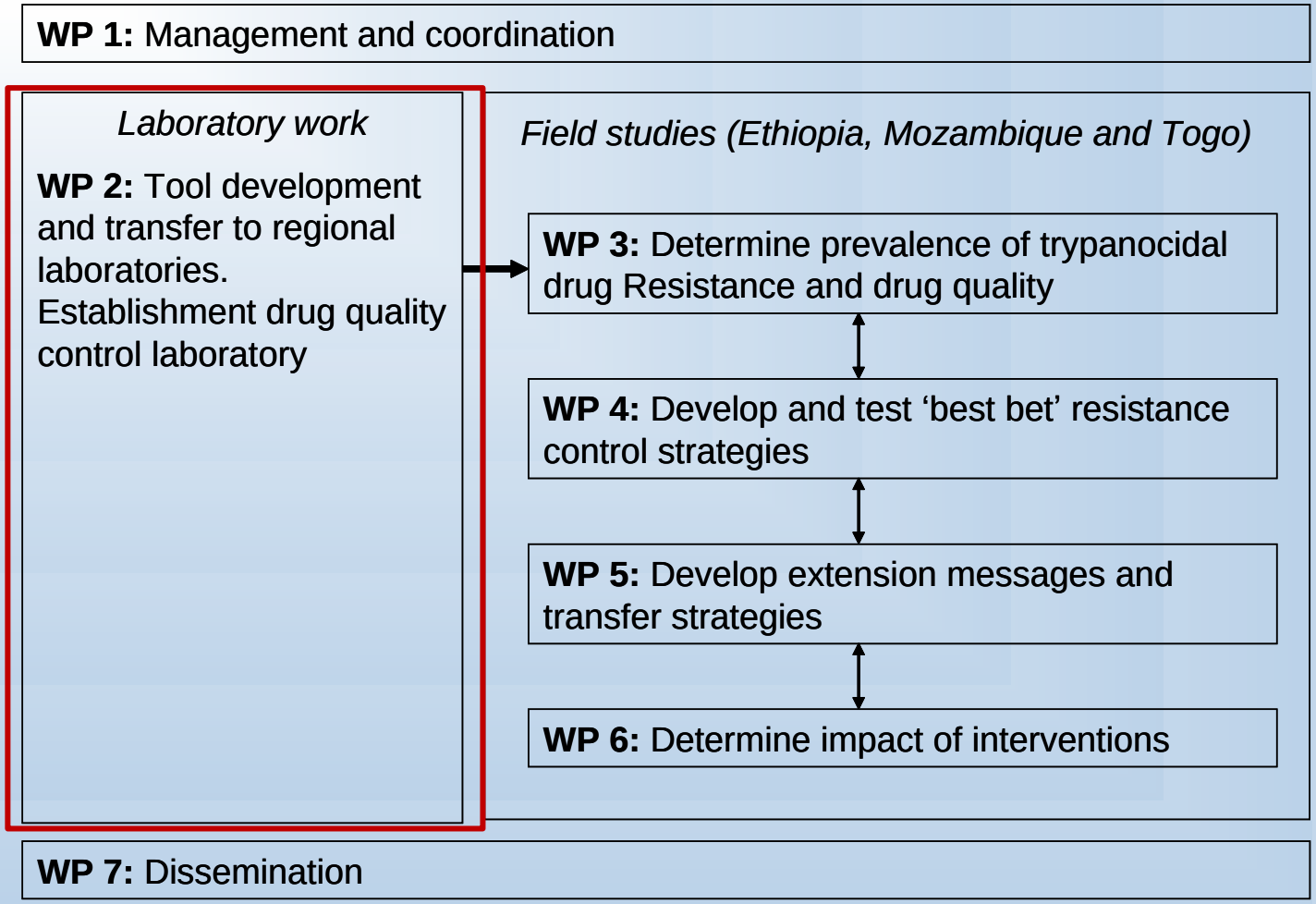
TRYRAC-WP2:

2nd PMC meeting- May 2013

Institute of Tropical Medicine Antwerp
Veterinary Protozoology Unit

WP 2: Overview



WP2: Objectives

1. Development of molecular tools to detect drug resistance
2. Transfer molecular tools and protocols to regional laboratories and control quality
3. Identify drug quality control laboratories

WP2: Molecular tools to detect drug resistance

- Focus on *T.congolense*
- Currently - two main drugs in use : Diminazene (DA) and Isometamidium-chloride (ISM)
- *In vivo* drug-resistance testing of field trypanosome strains: laborious; no large scale screening/monitoring; lab animal use; standardization.
- Molecular tool: exploiting genetic polymorphism that is linked to the drug-sensitivity phenotype; goal: 'easy-to-use' PCR-based assay
  - for DA: exists and has been validated
  - for ISM: non-existing

WP2: Molecular tools to detect DA-resistance

Based on the detection of a single nucleotide polymorphism (SNP) of the *TcoAT1* gene, a paralogue of the *T.brucei TbAT1/P2* aminopurine transporter gene (involved in the diamidine and arsenical drug uptake)

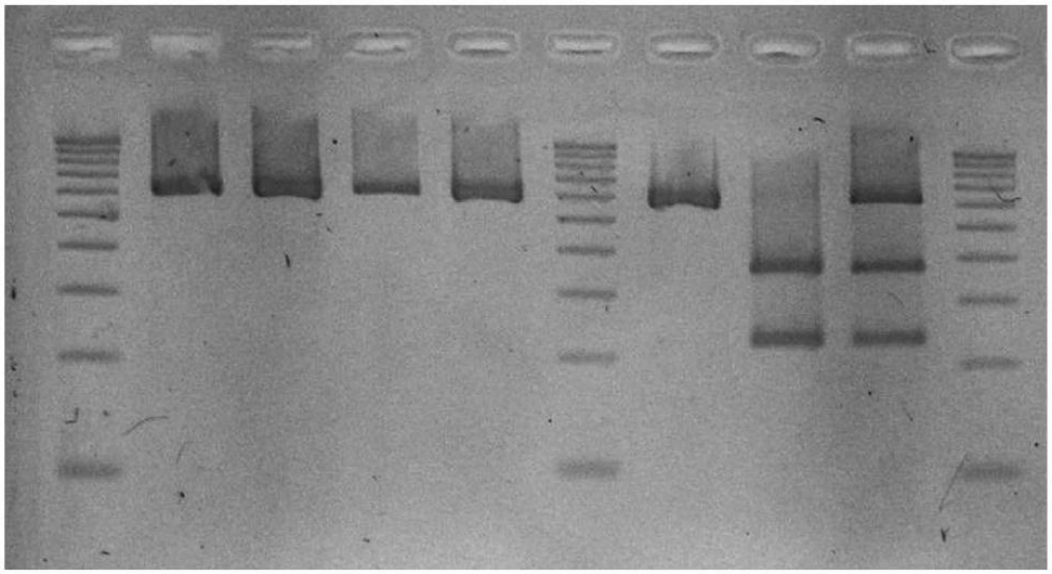


Figure 3. Output of the *DPn*II-PCR-RFLP with lanes 1, 6, and 10 as size markers (100-bp ladder), lanes 2, 3, 4, 5, and 7 as sensitive profiles (one band), lane 8 as resistant profile (two bands), and lane 9 as mixed profile (three bands).
doi:10.1371/journal.pntd.0001223.g003

Vitouleyh et al. (2011) PLoS NTD, 5, e1223

WP2: Molecular tools to detect DA-resistance

- However:

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Functional expression of *TcoAT1* reveals it to be a P1-type nucleoside transporter with no capacity for diminazene uptake ☆

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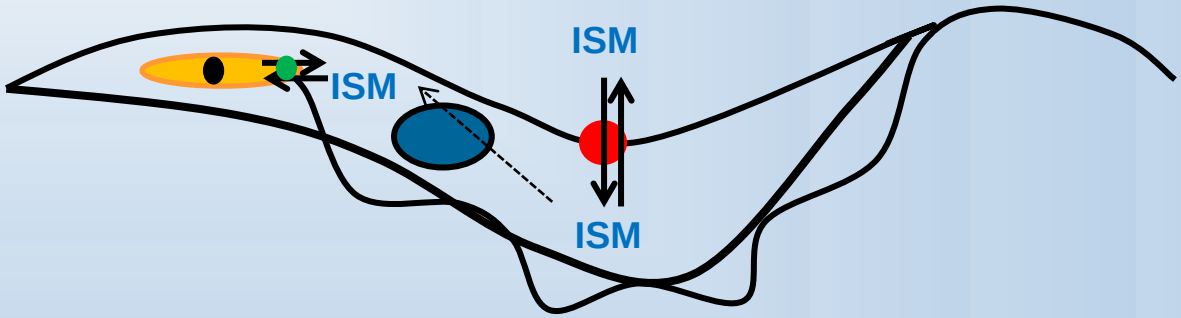
TcoAT1 does not mediate DA-uptake and is therefore unlikely to play a role in DA-resistance; *T. congolense TcoAT1* SNP is not directly linked with the DA-resistance phenotype → more research needed

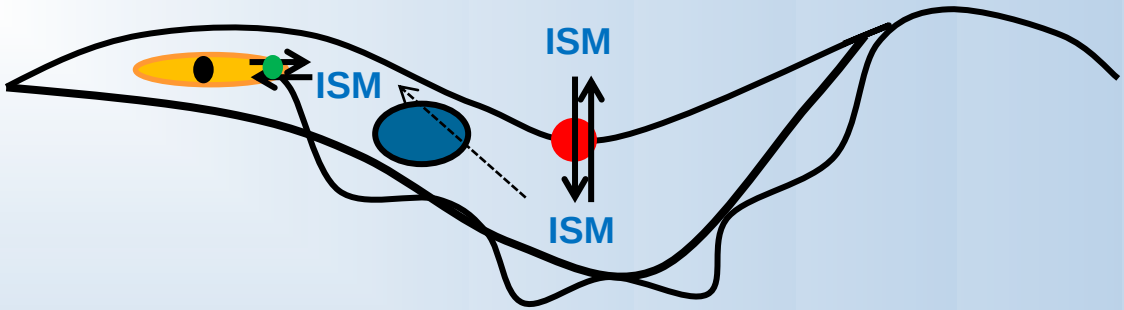
WP2: Molecular tools to detect ISM-resistance

- Molecular tool for Isometamidium-chloride resistance: does NOT exist → to be developed (possible?)



- Molecular mechanism of ISM uptake (at cell membrane and mitochondrium membrane level) ?
- Genetical basis /Molecular mechanism of ISM resistance?

WP2: Molecular tools to detect ISM-resistance



1. Differential 'omics approach:
- Genomic level
- Transcript level
- Protein level
- Metabolomic level

2. Functional experimental work

1. Lab-induced ISM resistance

2. Field isolates from cattle with treatment failure + confirmed *in vivo* resistance

WP2: Molecular tools to detect ISM-resistance

- Recruitment PhD-student:
  - International vacancy
  - 20 applications
  - Selection committee of 2 Profs & 5 Post-docs from VPU and MPU (department BMS-ITM)
  - → Eliane Tihon; started on 1 October 2012
  - → presentation PhD-work

WP2: Transfer of molecular tools

- Preparation labs (UP, CIRDES, NAHDIC) for transfer: UP and CIRDES are already well-equiped and have the hands-on expertise to perform molecular diagnosis through PCR.
- Tools to monitor DA-resistance are already installed in CIRDES and UP. This will be done in the coming months to NAHDIC. This laboratory performs PCR on a routine basis so that the transfer will be easy.

WP2: Drug quality control laboratories

- Candidate African laboratories were visited
- The laboratories for drug quality testing were identified: Dakar (LACOMEV) and Tanzania. Protocols were harmonized between the two laboratories by FAO and the Global Alliance for Veterinary Medicines (GALVmed). Drug quality analysis are available on demand (→ V. Delespaux).