Development of a mobile molecular lab (mLab) for the xenomonitoring of African trypanosomiasis

Background

Current field diagnostics for human African trypanosomiasis (HAT) were developed prior to the advent of molecular methods and although pragmatic are not suitable as monitoring tools checking for resurgence in near-elimination or post-elimination settings. As control of HAT intensifies and success is made the community has found that it lacks the diagnostics required to continue to accurately monitor the conditions on the ground. The goal of this project was to develop a fully mobile molecular laboratory (mLab) that can be used to screen the vector of African trypanosomiasis for the disease in low resource settings. These low resource settings would include locations with unreliable or non-existent electrical power supply, no dedicated laboratory and minimal access to postal or delivery service. As such the laboratory would have to be compact, be capable of supplying its own power and to operate for extended periods without re-supply. Key hurdles to be overcome include:

1) Suitable qPCR assays that are not reliant on a cold chain
2) Field friendly DNA isolation method that is rapid and simple but comparable to current tsetse DNA extraction methods
3) Trial the novel qPCR and DNA isolation methods in-country

Methods

A HRM qPCR assay was adapted from a nested-ITS PCR to screen field-tsetse for trypanosomes, with each major tsetse species identified based on their unique melt-peak signature. To compliment the ITS qPCR, a HAT specific qPCR was also included to provide the mobile laboratory the ability to discriminate between the sub-species of *T. brucei s.l.* and identify *T. b. gambiense* positive flies. This gives the mLab an important tool to help it monitor a post-elimination gambian-HAT foci. To negate the need for a cold chain to store reagents all qPCR assays were adapted to a dry-format, allowing for transportation and storage at room temperature.

Parallel to the new qPCR assays a DNA isolation method was developed utilising magnetic beads, allowing for rapid and simple DNA extraction and same-day qPCR. To confirm successful DNA isolation a novel internal control qPCR was developed which targeted the obligate symbiont *Wigglesworthia glossinidia* to confirm successful DNA isolation.

These methods were then introduced to field staff and trialled at the Trypa-NO! research field laboratory in Arua (N.W. Uganda). As part of the project field staff underwent an intensive 2 week training course to introduce them to the qPCR assays and the new hardware.

Results and Conclusions

The field-friendly mobile molecular laboratory were successfully introduced to the field site and has been running as a trial for ~3 months, in that time it has successfully processed 320 samples. The new DNA isolation method has consistently performed well, confirmed by the amplification of the *W. glossinidia* marker. The mLab also successfully detected several trypanosome species markers in the locally caught tsetse population.