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An LC-MS based metabolomics platform for *Leishmania*: development and evaluation.

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Comparative metabolomics of *Leishmania* species requires the simultaneous quantification and identification of a large number of intracellular metabolites. Here we present a comprehensive optimised protocol for metabolite extraction and subsequent analysis by mass spectrometry, specifically developed for *Leishmania donovani*. The protocol starts with rapid quenching of promastigotes to 0°C. After a triplicate washing step, the intracellular metabolome of 4 x 10^7 parasites is extracted in cold chloroform/methanol/water 20/60/20 (v/v/v) for 1h at 4°C, resulting in both cell disruption and comprehensive metabolite dissolution. Finally, metabolites are separated, identified and quantified by hydrophilic interaction liquid chromatography (HILIC) coupled to LTQ-Orbitrap mass spectrometry. This approach can detect approximately 20% of the predicted *Leishmania* metabolome in a single experiment using both positive and negative ionization mode.

The power of the optimised metabolomics protocol was further tested in 2 pilot-studies; each addressing a different aspect of the *Leishmania* biology. In a first pilot-study, 2 distinct *Leishmania donovani* phenotypes (drug sensitive and resistant) were compared, and the results demonstrate that untargeted metabolomics experiments can highlight entire metabolic pathways potentially involved in drug resistance mechanisms. The second pilot-study aimed to unravel the dynamics of both the intracellular and extracellular (culture medium) metabolome of *Leishmania* during 8 days of *in vitro* promastigote growth. The resulting metabolome profiles enabled us to map the dynamic changes of various metabolic pathways during the *in vitro* differentiation of promastigotes into infectious metacyclics.

The presented metabolic pipeline will be used routinely in a multinational initiative for the integration of diverse post-genomic technologies in *Leishmania* research, the GeMInI consortium (http://www.leishrisk.net/gemini), which brings together next generation sequencing and high-performance metabolomics on drug-sensitive and -resistant clinical *Leishmania donovani* isolates.