**The effect of formulation and radiation – a quantitative imaging-based analysis of sporozoite motility**

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**Introduction:** An effective malaria vaccine has the potential to prevent around half a million deaths each year. Live attenuated malaria parasites (sporozoites; spz) are considered the most promising vaccine candidates, however their efficacy critically depends on the spz potential to migrate in the human host. Key components of the spz motility machinery have been identified, however the regulation of this machinery is an unknown process. Moreover, (life attenuated) spz migration in human tissue (e.g. skin) remains wholly uncharacterized to date. We have developed the quantitative analysis tool SMOOT which enabled us to study spz motility *in vitro* and in human skin. Using this tool, we assessed how formulation and radiation affects sporozoite motility.

**Methods:** Fluorescence confocal microscopy was used to image *Plasmodium berghei* spz *in vitro* in nine different formulations to investigate the regulation of spz motility by the different components available in solution. Also, movies were obtained of wild type and radiation attenuated *Plasmodium falciparum* spz migrating in human skin explants to study spz migrating behaviour in the human host and to investigate how this is affected by radiation attenuation. SMOOT enabled the quantitative analysis of key spz motility features: their adherence capacity, movement pattern, directionality and velocity during forward locomotion.

**Results:** Image analysis revealed that spzmotility is highly dependent on the environment. *In vitro* spz displayed primarily (98%) counter clockwise circular movement. Albumin acted as an essential supplement to induce parasite attachment and movement. Glucose, salts and other whole serum components further increased the attachment rate and regulated the velocity of the movement. In human skin, the physical constraints asserted by the tissue morphology slowed down the spz and yielded more complex directional migration patterns; spz movement alternated between turning (either CW and CCW) and linear patterns. Head-to-head comparison revealed that radiation attenuation impaired the capacity of sporozoites to vary their movement angle and velocity, promoting less refined movement patterns.

**Conclusion:** We created a tool which enabled quantitative analysis of spz motility both *in vitro* and in human skin. We revealed that a complex interplay of albumin, glucose and certain salts and amino acids regulates parasite motility. Moreover, we showed that radiation attenuation altered spz migration behaviour. Insights in how parasite motility is regulated by formulation and affected by radiation attenuation will potentially contribute to the development of an efficacious live attenuated parasite based malaria vaccine.