Development of a novel sporozoite seroneutralization assay for ECF November 2019 – March 2021 Frozen stock of a Cell line or ConA Blast Serum at different concentrat ions Sporozoites from frozen batches at fixed dilution. X min at RT Incubate 1 to 3 days Infection (no need multiplication) P104 based qPCR

Objectives

- Overcome the limitations of the old sporozoite seroneutralization assay, such as the use of fresh sporozoites and cells, long incubation periods and a difficult and time consuming read-out.
- Generate an absolute quantification read-out for the assay using qPCR on p104 T. parva ORF. Quantitative assay, not only qualitative.
- 3) Reduction on animal usage in sporozoite subunit vaccine research.

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Funding: TAHSSL - BMGF

Highlight of 2020 achievements

- Generation and validation of sporozoite new frozen batches.
- Validation of T-cell CD4 ConA Blast as possible cell biobank to use in the assay.
- Set-up and validation of p104 qPCR with plasmid standard for absolute quantification of equivalent genome copies, as read-out of the sporozoite seroneutralization assay.