

The *P. vivax* liver stage *in vitro* assay should at least fulfill the following criteria:

- Format: 96 or 384 well plate assay
- Demonstration that a good rate of infection (minimum of 1% infection rate, ideally 5%) is achievable and robustly reproducible over time ($z' > 0.5$)
- Demonstration of regular production of biomass necessary to run the assay every month or more frequently: *P. vivax* sporozoites, primary hepatocytes or hepatocytic cell line
- Ability to produce long term liver stage cultures (up to 9-12 days)
- Macroscopic validation of the presence of both schizont forming parasites (developing large exo-erythrocytic forms) and small non-developing exo-erythrocytic forms (sEEF) morphologically proven to be similar to hypnozoites
- Chemical validation of mature sEEF as hypnozoites with atovaquone and primaquine (or primaquine metabolites in case the cell line used is non-metabolically active)
- Ideally observation of relapse from those sEEF *in vitro*
- Cost: Minimum <5\$ per well, ideally <1\$ per well
- An assay gathering all the above criteria but using the *P. vivax* closely related strain *P. cynomolgi* will be of interest

The *P. vivax* liver stage *in vivo* assay should at least fulfill the following criteria:

- The model needs to be performed in mice
- Demonstration that a good rate of infection is achievable and robustly reproducible over time
- Method to measure and detect hypnozoites present in the liver is in place and robust
- Chemical validation of sEEF as hypnozoites with *in vivo* dosing of both atovaquone and primaquine