

## STANDARD OPERATING PROCEDURES

**TITLE:** THE HRP2 DRUG SENSITIVITY ASSAY FOR LAB USE

**SOP ID Number:** ETM-002

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**Purpose and Scope:** This SOP describes the HRP2 drug sensitivity assay for use with culture adapted *P. falciparum* strains or clones and the handling of fresh samples in well equipped laboratories (please see SOPs for the “field test” for the testing fresh samples in the field).

**Definitions:** NA

**Safety Concerns:** All safety precautions related to the handling of potentially infectious human and parasite material apply.

### Specific Materials and Equipment:

- 96-Well Microculture plates precoated with antimalarial drugs: e.g. Costar 3599 or Falcon 3070. Caveat: adjust volume/concentration for fresh vs. dried plates.
- Complete RPMI 1640 Medium (10.43g RPMI 1640 powder + 6 g HEPES + 25 mg gentamycin + distilled water to 1L). Add NAHCO<sub>3</sub> (2.8 ml of 7.5% NAHCO<sub>3</sub> per 100ml medium before use)
- Serum (blood group AB or same as RBCs)
- RBCs (blood group 0 or same as serum)
  
- Multichannel pipette (20-200 uL)
- One set of adjustable pipettes
- Sterile trays
- Incubator
- Candle jar or incubation chamber with gas mixture
- Freezer (-20 °C or below)

### Specific Procedures:

**1.) Sample preparation and culture:** the HRP2 assay requires a 72 hour incubation time to obtain a distinct increase in HRP2-concentrations in the controls. Culture procedures are similar to those in other drug sensitivity assays.

- Parasitized blood samples (e.g. from continuous culture) are diluted with uninfected red blood cells (blood group O) to obtain 0.05% parasitemia of >80% ring stage parasites and with RPMI 1640 medium (with 10% heat inactivated serum of the same blood group or blood group AB) to 1.5% hematocrit. Parasites

- may be synchronized using. Make thick/thin film to verify initial parasitemia.
- Add 200 uL of the resulting cell medium mixture to each well of the predosed plates (start with well A and proceed to higher drug concentrations). Use a number of drug-free wells to obtain additional control samples to check for parasite growth after the incubation.
  - Incubate the plates for 72 hours at 37°C in a candle jar or in a gas mixture consisting of 5% CO<sub>2</sub>, 5% O<sub>2</sub>, and 90% N<sub>2</sub>.
  - Regas and carefully shake the plates after 24 and 48 hrs.
  - After 72 hrs prepare another slide (thin film) to count parasites. Adequate growth should lead to a 4 to 10 fold increase in parasite density.

**2.) Hemolyzing:** after the end of the incubation time (72 hrs) the samples are hemolyzed by freeze-thawing.

- The plates are removed from the incubator and separately transferred into a freezer, where they remain until all wells are completely frozen (preferably over night - depending on the temperature this procedure should take around 60 minutes or longer).
- Thaw the plates (use an incubator to speed up thawing). Repeat the procedure at least once until complete hemolysis is achieved (i.e. all wells look completely clear).

### References:

Noedl H, Wernsdorfer WH, Miller RS, Wongsrichanalai C. Histidine-rich protein II: a novel approach to malaria drug sensitivity testing. *Antimicrob Agents Chemother.* 2002 Jun;46(6):1658-64.