

STANDARD OPERATING PROCEDURES

TITLE: THE HRP2 DRUG SENSITIVITY ASSAY FOR FIELD USE

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Purpose and Scope: This SOP describes a simplified HRP2 drug sensitivity assay for field use which is optimized as to require minimum infrastructure and technical equipment while providing the highest possible levels of sensitivity. This SOP is designed for use with fresh *P. falciparum* isolates (field samples) only (see corresponding SOPs for testing of culture adapted strains and clones).

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 + plus 0.5% w/v of Albumax I + distilled water to 1L). Add NAHCO₃ (2.8 ml of 7.5% NAHCO₃ per 100ml medium before use)
- RBCs (blood group 0 or same as serum)
- Sterile disposable phlebotomy tool
- 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 collection:

- Identify patients with *P. falciparum* mono-infections with parasite densities of **0.002 % or greater** (approx. 100 parasites per uL or more). Samples with parasite densities of 1% or more may be used but should be diluted with uninfected RBCs to obtain a density of approximately 0.2% before testing to limit the inoculum effect.

- After thorough disinfection of the skin collect a minimum of 1 mL of blood by venepuncture using a sterile disposable phlebotomy tool and a heparinized container.
- Prepare thick and thin blood films, thoroughly dry the slides, fix thin films with methanol, and stain with Giemsa (3%, 20 mins), microscopically examine the slides (oil, 1000x magnification) and assess and record the parasitemia (preferably in thin film).

2.) **Sample preparation and culture:** the HRP2 drug sensitivity field test uses 72 hours of incubation at 37 °C.

- To obtain a total of 25 mL (for one culture plate) of cell medium mixture add **24.06 mL** of complete RPMI 1640 medium (10.43g RPMI 1640 powder + 6 g HEPES + 25 mg gentamycin + plus 0.5% w/v of Albumax I + distilled water to 1L and add NaHCO_3 (2.8 ml of 7.5% NaHCO_3 per 100ml medium before use)) to a sterile disposable tube (medium filled tubes may be prepared in advance and stored at 4°C for several days).
- Add **0.94 mL** of the parasitized blood to the tube to obtain a cell medium mixture (CMM) with approximately 1.5% hematocrit (assuming a 40% hematocrit in the parasitized blood sample).
 - Alternatively wash and dilute the original parasitized blood sample with uninfected RBCs to 0.05% and 1.5% htc before adding it to the medium (this way the dilution of the samples before the ELISA may be omitted). The advantage of this procedure is that this way the inoculum effect may largely be excluded.
- Add 200 µL of the resulting CMM to each well of the predosed plates (start with well A and proceed to higher drug concentrations).
- Incubate the plates for 72 hours at 37°C in a candle jar or in a CO_2 -enriched gas mixture consisting of 5% CO_2 , 5% O_2 , and 90% N_2 .
- After the end of the incubation time (72 hours) the plates may be further processed immediately (if an ELISA plate reader is available), or stored preferably at or below -20°C and transported to the laboratory facilities for the ELISA (a simple household freezer is usually also adequate). After 72 hrs prepare another slide to determine parasitemia. Adequate growth should lead to a 4 to 10 fold increase in parasite density within 72 hrs.

3.) **Hemolyzing:** after the end of the incubation time the samples are harvested and frozen-thawed.

- 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. If not completely haemolysed repeat the procedure at least once until complete haemolysis is achieved (i.e. all wells look completely clear).

References:

Noedl H, Attlmayr B, Wernsdorfer WH, Kollaritsch H, Miller RS. A histidine-rich protein 2-based malaria drug sensitivity assay for field use. *Am J Trop Med Hyg.* 2004 Dec;71(6):711-4.