Preparation of predosed plates v1.2

Procedure

In vitro Module

WorldWide Antimalarial Resistance Network (WWARN)

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Version History

Version number	Revision(s) & reason for amendment	Release Date
1.0	Creation of the document	16/08/2010
1.1	Changes to the template	29/11/2010
1.2	Changes to Sections 6.2 & 6.3	01/04/2011

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Contents

1. Purpose

This procedure describes the preparation of pre-dosed and freshly made plates for use with culture-adapted *P. falciparum* clones or fresh samples in drug sensitivity assays.

2. Scope

This procedure is designed for use by laboratories testing *in vitro* drug susceptibility using *P. falciparum*. This procedure is applicable to well-equipped cell culture laboratories. Considerable training is required to perform the procedure successfully.

3. Abbreviations

P. falciparum	*Plasmodium falciparum*
RPMI	Roswell Park Memorial Institute medium 1640

4. Duties and Responsibilities

The preparation of the predosed plates must be carried out by a competent technician.

5. Materials and Equipment

5.1 Materials

- Sterile graduated pipettes
- Sterile vials
- 12 channel multipipette
- Clear, cell culture-treated 96-well microtiter plates
- 1mL serological pipettes
- Sterile micropipette tips
- Volumetric flask
- Ethyl alcohol
- Methanol
- Sterile pure water
- Standardized antimalarials pre-weighed in vials:
    - Dihydroartemisinin
    - Piperaquine
    - Lumefantrine

- Desethylamodiaquine
- Chloroquine
- Mefloquine
- Quinine
- Pyronaridine
- Doxycycline
- Atovaquone

5.2 Equipment

- Laminar flow hood.
- Cryogenic equipment at + 4 °C
- Cryogenic equipment at - 20 °C

6. Procedure

6.1 Preparation of stock solution

Each test compound is packaged as a powder in a sterile vial. Vials are stored at +4 °C. Dissolve drug powder in the volume of solvent according to required drug range to obtain stock solution concentration. Appendix A notes the appropriate solvent and storage instructions for the stock solution

NOTE: Stock solutions have a shelf life of up to 1 month when stored at +4 ºC. They can be successfully stored as aliquots at -80 ºC with increased shelf-lives.

6.2 Preparation of working solution

Prepare working solutions by further diluting the stock solution in the appropriate solvent. The final dilution should be matched against the highest concentration of drug in the *in vitro* test plate.

6.3 Preparation of plates

6.3.1. Dry plates
A batch of plates can be prepared, dried and stored for later use.

I. Prepare the required range of reference drug concentrations by diluting stepwise 1 in 1.5, or 1 in 1.25 (depending on the compound under test) 21 times.

II. When the dilutions are finished, use a 12 channel multipipette to dispense one row at a time with either 25µl (200µL final volume culture) or 12.5µL (100µL final volume of culture) of drug dilution into each well of a sterile 96-well microplate.

III. The last row should not contain any drug dilution as it will be used as the control.
IV. The test plates must be thoroughly air-dried before use (for several hours to overnight) in a sterile environment.
V. Protect pre-dosed plate with a film or a cover.
VI. Store plates at +4 °C until ready for use.

6.3.2. Fresh plates
Plates can be prepared fresh and used within the day.

I. In tubes, prepare a twofold serial dilution of the drug final working solution. The dilutions are made in complete medium for malaria culture and mixed thoroughly by aspiration.
II. Use a 12 channel multipipette to dispense, one row at a time, 25µl of drug dilution into each well of a sterile 96-well microplate.
III. The last row does not contain any drug dilution as it will be used as the control.
IV. Protect plates with a film or a cover.
V. Store plates at +4 °C and use them within the day.

6.4. Quality control

Each batch should be validated against reference clone 3D7 to assure that drug quality was maintained over the period of use.

Validation criteria should be assessed by each laboratory as they vary depending on the *in vitro* drug susceptibility testing method used.

QC records should be retained and approved. Uniformity of plate batches must be assessed using:

- Clones
- Drug susceptibility values.

7. References

Basco LK., *Field application of in vitro assays for the sensitivity of human malaria parasites to antimalarial drugs.* World Health Organization. Available from: http://www.who.int/malaria/publications/atoz/9789241595155/en/index.html (Accessed: 29 November 2010)

Houze S. *et al*., *Shelf Life of predosed plates containing mefloquine, artemisinin, dihydroartemisinin and artesunate as used for in vitro P. falciparum susceptibility assessment*. Journal of Clinical Microbiology. Aug 2007: 2734-2736

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Appendix A: Stock solutions, solvents and dry plate shelf lives

Molecules	Solvents for dissolve powder	Solvents for dilutions	Final concentrations (nM)	Plates Shelf lives
Dihydroartemisinin	methanol	Water	0.15 – 63	1 month
Piperaquine $4H_3PO_4$ $4H_2O$	Lactic acid 0.5%	Water	1.9 – 801	2 months
Lumefantrine	Absolute ethanol	Absolute ethanol	0.74 – 311	3 months
Desethylamodiaquine	Methanol	Water	4 – 1912	1 year
Chloroquine $2H_3PO_4$	water	water	8 – 3200	1 year
Mefloquine HCl	methanol	water	4 – 401	2 months
Quinine HCl	methanol	water	30 – 3209	1 year
Pyronaridine $4H_3PO_4$	water	water	1.25 – 80	2 months
Doxycycline (µM)	methanol	Water	1.2 – 501	1 month
Atovaquone	methanol	Water and methanol	0.9 – 40000	1 month