Culture of *Plasmodium falciparum* blood stages v1.0

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 procedure	16/08/2010

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Contents

1. Purpose

This procedure describes the conditions and method of culture for *P. falciparum* clones or fresh isolates. Clone ratios (3D7) permit the validation and the standardisation of data from different *in vitro* testing methods done in different settings and at different times.

2. Scope

This procedure is designed for use by a laboratory working on *in vitro* drug sensitivity testing methods 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
FW	Formula weight

4. Duties and Responsibilities

The culture of *P. Falciparum* erythrocytic stages must be carried out by a competent technician.

5. Materials and Equipment

5.1 Materials

- RPMI medium for washing
- Complete medium for the culture of *P. falciparum* (RPMI medium supplemented with 10% serum)
- Uninfected erythrocytes (blood group O)
- Sodium chloride
- Freshly prepared Giemsa stain
- 25 cm^2 sterile plugged sealed tissue culture flasks
- Sterile propipettes
- Sterile graduated pipettes

- Disposable microscope slides

5.2 Equipment

- Cryogenic equipment at 4° C
- Cryogenic equipment at –20° C
- Cryogenic equipment at –196° C
- Laminar flow hood
- Incubator with a reliable source of CO_2 or incubation chamber with gas mixture or candle jar
- Microscope with a 100x oil immersion objective
- Centrifuge
- Water-bath or heater block
- Shaker

6. Procedure

6.1 Preparation of red blood cells

Red blood cells are used in dilutions of culture to lower parasitemia and maintain hematocrit.

I. Warm RPMI medium to 37° C in water-bath or heater block.
II. Centrifuge red blood cells at 500 g for 5 minutes.
III. Add 2:1 v/v RPMI medium to erythrocytes pellet to return to the original volume.
IV. Stir 3–5 minutes at room temperature on a shaker.
V. Centrifuge at 500 g for 5 minutes.
VI. Remove supernatant.
VII. Wash the pellet of packed red cells two more times as above.

6.2 Preparation of the sample

6.2.1 Clones
Clones are stored in glycerolyte at –196° C in liquid nitrogen.

I. Prepare a sterile NaCl solution at 3.5%. Conserve at 4° C.
II. Warm RPMI medium to 37° C in water-bath or heater block.
III. Thaw the frozen sample at room temperature in 1–2 minutes.

IV. Transfer the contents to a new sterile tube.

V. Add NaCl solution drop by drop, very slowly, inverting gently each time and waiting for a minute or two. This slow timing is crucial to avoid lysis of the red cells and will take 5–10 minutes.

VI. Agitate gently 3–5 minutes at room temperature on a shaker.

VII. Centrifuge at 500 g for 5 minutes.

VIII. Remove supernatant.

IX. Wash the pellet two more times with RPMI medium 1:9 v/v.

6.2.2 Isolates

There is a possibility for short term culture of patient isolates before running an *in vitro* test but this may select clones. Isolates must be washed three times in RPMI and the white cells removed. An isolate is successfully adapted in continuous culture if there is a more than five-fold increase in growth per cycle.

6.3 Culture and culture condition

I. Warm complete medium to 37° C in water-bath or heater block.

II. In a flask of 25 cm^2, add 8 mL of complete medium.

III. Add packed uninfected erythrocytes to packed infected erythrocytes as to obtain a total volume of 500 µL.

IV. Add this volume to the medium to obtain a 5% hematocrit.

V. Stir gently.

VI. Maintain the culture of *P. falciparum* in candle jar or in incubator in the following conditions:

- temperature: 37° C
- gas mixture: 5% CO_2, 5–10% O_2 and 85–90% N_2
- humidity: > 90%

6.4 Long-term culture

6.4.1 Medium renewal

The culture medium has to be changed every 24 hours in a sterile environment.

I. With a sterile graduated pipette, withdraw the medium above sedimented cells. Note the amount removed volume.

II. Add the same volume of fresh medium to the flask.

6.4.2 Control of parasitemia
At every medium renewal, parasitemia must be assessed.

I. Make thin blood films.

II. Stain with Wright's stain or Giemsa method.

III. By microscopic examination:

- determine the parasitemia
- assure that there is no contamination

IV. If the parasitemia exceeds 5%:

- centrifuge at 500 g for 5 minutes
- discard the supernatant
- dilute the pellet with uninfected red blood cells to obtain a 0.5–1% parasitemia and with complete medium to 5% hematocrit.

6.5 Short term culture for *in vitro* testing

The first stage in the *in vitro* drug sensitivity assays is incubation of the culture. In each method, the culture must be diluted to the value specified according to the corresponding procedure for preparing the plate for the test.

I. Centrifuge at 500 g for 5 minutes.

II. Discard the supernatant.

III. Dilute the pellet with uninfected red blood cells to obtain the required parasitemia and with complete medium to 1.5% hematocrit.

6.6 Synchronisation

To measure drug sensitivity, clones must be synchronised in the ring stage.

6.7 Quality control

QC records must be kept and approved. Preparation and sterility of red blood cells and NaCl solution must be assessed by:

- preparation date
- powder weights
- reagent batch numbers

7. References

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