Synchronisation of *Plasmodium falciparum* v1.1

Procedure

In vitro Module

WorldWide Antimalarial Resistance Network (WWARN)

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This procedure was developed by:

In vitro Module, WWARN

Version History

Version number	Revision(s) & reason for amendment	Release date
1.0	Creation of the procedure	16/08/2010
1.1	Changes to the template	29/11/2010

For more information, contact:
invitro@wwarn.org

WorldWide Antimalarial Resistance Network (WWARN)
www.wwarn.org

Contents

1. Purpose

This procedure sets out the method to be used for the synchronisation of culture adapted *P. falciparum* clones in ring stage. Ring forms of at least 95% are required for drug sensitivity assays. 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 laboratories 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. Competency may be assessed by close observation by an approved trainer.

3. Abbreviations

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

4. Duties and Responsibilities

The synchronisation of *P. falciparum* must be carried out by a competent technician.

5. Materials and Equipment

5.1 Materials

- RPMI medium for washing
- Percoll
- PBS
- D-sorbitol
- Water for cell culture or or bidistillated water
- 25 cm^2 sterile plugged seal tissue culture flasks
- Sterile propipettes
- Sterile graduated pipettes
- Disposable microscope slides
- 100 mL volumetric flask
- Cups and spatula
- Disposable sterilisation filter unit and filter unit receiver
- Sterile vials.

5.2 Equipment

- Cryogenic equipment at 4° C
- Laminar Flow hood
- Vacuum trap
- Incubator with a reliable source of CO_2 or candle jar
- Microscope with a 100x oil immersion objective
- Centrifuge
- Water-bath or heater block
- Shaker
- Densitometer.

6. Procedure

In a sterile environment:

6.1 First stage: schizont forms selection

Parasitemia of the culture must be assessed by making a coloured thin blood film. If at 5% parasitemia, schizont forms represent the majority, it can be separated from parasites in other stages.

6.1.1 Percoll solution preparation

I. Add 8 mL of 10X PBS in 17.5 ml of RPMI 1640.

II. Add 72 mL of Percoll.

III. Stir gently to obtain a homogeneous solution.

IV. Measure the density of the mix and adjust it at 1.085:

- by adding RPMI 1640 if the density > 1.085
- by adding Percoll if the density < 1.085.

NOTE: Percoll increases density.
If Percoll is used for adjustment, add PBS to maintain a 9 v/v Percoll/PBS ratio.

V. In a sterile environment, filter sterilise medium with 0.22 µM filter.

VI. Dispatch 4 mL of solution in 5 mL tubes.

VII. Store at 4° C. The solution is good for 3 months.

6.1.2 Schizont forms sedimentation

I. Warm a tube of Percoll solution to 37° C in water-bath or heater block.

II. Centrifuge the culture at 500 g for 5 minutes.

III. Discard supernatant and stir the cell pellet.

IV. With a sterile pipette, collect the cell pellet.

V. Gently lay down the collected blood at the surface of Percoll solution.

VI. Centrifuge the tube at 1300 g for 15 minutes.

VII. With a sterile graduated pipette, withdraw the ring created by schizonts forms in the superior phase of solution.

VIII. Add 1:9 v/v RPMI medium to cell supernatant.

IX. Stir 3–5 minutes at room temperature on a shaker.

X. Centrifuge at 500 g for 5 minutes.

XI. Remove supernatant.

XII. Wash packed red blood cells two more times.

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

XIV. Add washed infected cell pellet.

XV. Complete to 400 µL with uninfected erythrocytes to obtain a 5% hematocrit.

XVI. Stir gently.

6.2 Second stage: culture

Maintain the culture of *P. falciparum* in candle jar or in incubator for 6 hours in conditions determined in procedure culture of *P. falciparum*.

NOTE: An alternative method to enrich a culture in schizonts is to use a magnetic separation kit.[1]

6.3 Third stage: ring forms selection

At this stage, parasitemia must be assessed by making a coloured thin blood film.

- If ring % > 95%, the drug sensibility of the culture can be assessed directly.
- If ring % < 95%, proceed with the next step as ring forms are not sufficient.

6.3.1 Sorbitol solution preparation

I. Weigh 5 g of D-sorbitol.

II. Dissolve sorbitol with cell culture water in a 100 mL volumetric flask.

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[1] Ribaut C *et al.*, Concentration and purification by magnetic separation of the erythrocytic stages of all human Plasmodium species. *Malaria Journal* 2008 Mar 5; 7:45. Abstract available from: http://www.ncbi.nlm.nih.gov/pubmed/18321384 (Accessed 29 November 2010).

III. In a sterile environment, filter sterilised medium with 0.22 µM filter.

IV. Dispatch 3 mL of solution in 5 mL tubes.

V. Store at 4° C. The solution is good for 3 months.

6.3.2 Ring forms selection

I. Warm a tube of sorbitol solution to 37° C in water-bath or heater block.

II. Centrifuge the culture at 500 g for 5 minutes.

III. Discard supernatant and stir the cell pellet.

IV. Add 5:1 v/v sorbitol to cell pellet.

V. Stir 5 minutes at room temperature on a shaker.

VI. Wash one more time with RPMI medium.

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

VIII. Add washed infected cell pellet.

IX. Complete to 400 µL with uninfected erythrocytes to obtain a 5% hematocrit.

X. Stir gently.

XI. Maintain the culture of *P. falciparum* in candle jar or in an incubator for 2 (3) hours.

6.4. Quality control

Quality control records must be kept and approved by a competent person.
Preparation and sterility of solution must be assessed by:

- Preparation date
- Powder weights
- Osmolarity
- Density.

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).

SOP Centre National de Référence du Paludisme, Paris, France.

SOP Institut de médecine tropicale du Service de santé des armées, Marseille, France.