Synchronisation of *Plasmodium falciparum* v1.1

Procedure

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This procedure was developed by:

In vitro Module, WWARN

Version History

<table>
<thead>
<tr>
<th>Version number</th>
<th>Revision(s) &amp; reason for amendment</th>
<th>Release date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Creation of the procedure</td>
<td>16/08/2010</td>
</tr>
<tr>
<td>1.1</td>
<td>Changes to the template</td>
<td>29/11/2010</td>
</tr>
</tbody>
</table>

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Contents

1. Purpose ....................................................................................................................... 4
2. Scope .......................................................................................................................... 4
3. Abbreviations ............................................................................................................ 4
4. Duties and Responsibilities ....................................................................................... 4
5. Materials and Equipment ......................................................................................... 4
  5.1 Materials .................................................................................................................. 4
  5.2 Equipment ............................................................................................................... 5
6. Procedure .................................................................................................................... 5
  6.1 First stage: schizont forms selection ................................................................. 5
  6.2 Second stage: culture ............................................................................................ 6
  6.3 Third stage: ring forms selection ................................................................. 6
  6.4. Quality control ...................................................................................................... 7
7. References .................................................................................................................. 7
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 bidistilled 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 CO₂ 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 cm², 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.

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 cm², 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


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