Preparation of complete medium for malaria culture v1.2

Procedure

Procedure ID: INV02

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 document</td>
<td>16/08/2010</td>
</tr>
<tr>
<td>1.1</td>
<td>Changes to Sections 6.2 and 6.3</td>
<td>01/04/2011</td>
</tr>
<tr>
<td>1.2</td>
<td>Changes to template</td>
<td>31/08/2012</td>
</tr>
</tbody>
</table>

For more information, contact:
invitro@wwarn.org

WorldWide Antimalarial Resistance Network (WWARN)
www.wwarn.org
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. Procedures .......................................................................................................................5
   6.1 5% sodium bicarbonate solution ............................................................................5
   6.2 RPMI washing medium .........................................................................................5
   6.3 Complete medium ....................................................................................................6
   6.4 Quality control (QC) ..............................................................................................6
7. References ......................................................................................................................7
1. Purpose

This protocol describes the preparation of medium used to culture *P. falciparum* isolates or clones.

2. Scope

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

3. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute medium 1640</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>FW</td>
<td>Formula weight</td>
</tr>
</tbody>
</table>

4. Duties and Responsibilities

The preparation of complete medium for malaria cultures must be carried out by a competent technician.

5. Materials and Equipment

5.1 Materials

- Sterile graduated pipettes
- 100mL volumetric flask
- 1L volumetric flask
- Cups and spatula
- Disposable 0.22μM sterilisation filter unit and filter unit receiver
- Sterile vials
- RPMI 1640, powdered medium, stored at 4°C
- Sodium bicarbonate, NaHCO₃, FW 84.01, stored at RT
- HEPES, C₅H₁₀N₂O₄S, FW 238.1, stored at RT
- Hypoxanthine, C₅H₆N₄O, FW 136.11, stored at RT
- L-glutamine, C₅H₁₀N₂O₃, FW 146.1, stored at RT
- Glucose, C₆H₁₂O₆, FW 180.16, stored at RT
- Orotic acid, C₅H₆N₂O₄, FW156.1 (optional), stored at RT
- Gentamicin (optional), C₂₁H₄₃N₉O₇, FW 477.596, stored at -20°C
• Sterile human serum stored at -20 °C or Albumax stored at + 4 °C, protected from extended exposure to light
• Hydrochloric acid 1N
• Sodium hydroxide 1N
• Water for cell culture or double-distilled water

5.2 Equipment
• Analytical balance
• Magnetic stir plate
• Laminar flow hood
• Vacuum trap
• Cryogenic equipment at + 4 °C
• Cryogenic equipment at – 20 °C
• Water-bath or heater block
• pH meter
• Osmometer

6. Procedures

6.1 5% sodium bicarbonate solution
I. Weigh 5g sodium bicarbonate
II. Add 100mL of cell culture water in volumetric flask
III. Stir on magnetic stirring plate until dissolved
IV. Store at room temperature
V. Use within 24 hours of preparation

6.2 RPMI washing medium
Used to wash samples and red blood cells.
I. Weigh 10.43g of powdered RPMI medium and add to 1L volumetric flask
II. Add 5.95g HEPES (25mM final concentration)
III. Add 42mL 5% sodium bicarbonate (as prepared in 6.1) (25mM final concentration)
IV. Fill to 1L with cell culture water
V. Stir the solution until dissolved
VI. Using a calibrated meter, check medium pH. If necessary, adjust to pH7.0 ± 0.3 with either hydrochloric acid (1N) or sodium hydroxide (1N). The medium should be orange, not red/purple or yellow, in colour due to the phenol red dye
VII. Using a calibrated meter, check that the medium osmolarity = 292 ± 5% mOsm. If necessary, adjust with sterile water
VIII. In a sterile environment, use a 0.22μM filter to sterilise the medium
IX. Aseptically dispense medium into sterile bottles
X. Store at – 20 °C for a maximum 6 months
XI. After first use, store at + 4 °C for one week
6.3 Complete medium

Used to culture *P. falciparum* isolates or clones.

I. Weigh 10.43g of powdered RPMI 1640 medium (RPMI 1640 with L-glutamine) into a 1L volumetric flask

II. Add 5.95g HEPES (25mM final concentration)

III. Add 42mL 5% sodium bicarbonate (as prepared in 6.1) (25mM final concentration)

IV. Add:
   a. 50mg hypoxanthine
      **Note**: Hypoxanthine should **not** be added if culturing *P.falciparum* for ³H-hypoxanthine uptake inhibition assays *(INV07)*
   b. 430mg L-glutamine (0.3 g/L)
   c. 1g glucose
   d. 0.25mg orotic acid (0.25 mg/L; optional)
   e. 10mg gentamycin or 32mg neomycin (10 mg/L; optional)

V. Adjust volume to 1L with cell culture water

VI. Stir until dissolved

VII. Using a calibrated meter, check medium pH. If necessary, adjust to pH 7.0 ± 0.3 with either hydrochloric acid (1N) or sodium hydroxide (1N). The medium should be orange, not red/purple or yellow, in colour due to phenol red dye

VIII. Using a calibrated meter, check osmolarity = 292 ± 5% mOsm. If necessary, adjust with sterile water

IX. In an aseptic environment, sterilize the medium using a 0.22μM filter

X. Add sterile human serum to a final concentration of 10% or AlbuMAX™ I (lipid-enriched bovine serum albumin) to a final concentration of 0.5%.

XI. Aseptically dispense medium into sterile bottles

XII. Store at – 20 °C for a maximum 6 months

XIII. After first use, store at 4 °C for one week

XIV. Protect bottles from extended exposure to light

6.4 Quality control (QC)

Record:

I. Preparation date

II. Recorded powder weights

III. Reagents’ batch numbers

IV. pH

QC records should be approved by a competent person and stored in a safe location.
7. References


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

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

SOP Institut Pasteur du Cambodge.

SOP Institut Pasteur de Guyane.

SOP Institut Pasteur de Madagascar.