Preparation of complete medium for malaria culture 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 Sections 6.2 and 6.3	01/04/2011
1.2	Changes to template	31/08/2012

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Contents

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

RPMI	Roswell Park Memorial Institute medium 1640
RT	Room temperature
FW	Formula weight

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, $NaHCO_3$, FW 84.01, stored at RT
- HEPES, $C_8H_{18}N_2O_4S$, FW 238.1, stored at RT
- Hypoxanthine, $C_5H_4N_4O$, FW 136.11, stored at RT
- L-glutamine, $C_5H_{10}N_2O_3$, FW 146.1, stored at RT
- Glucose, $C_6H_{12}O_6$, FW 180.16, stored at RT
- Orotic acid, $C_5H_4N_2O_4$, FW156.1 (optional), stored at RT
- Gentamicin (optional), $C_{21}H_{43}N_5O_7$, 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 ^{3}H-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 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 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

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

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