Isolation of viable Plasmodium falciparum merozoites

Michelle Boyle  
Burnet Institute

Danny Wilson  
University of Adelaide

James Beeson (james.beeson@burnet.edu.au)  
Burnet Institute

Method Article

Keywords: Malaria, merozoites, host cell invasion, immunity, infection, merozoites

Posted Date: January 5th, 2024

DOI: https://doi.org/10.21203/rs.3.pex-2508/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

*P. falciparum* merozoites are the parasite form that infected red blood cells during blood-stage infection and replication. This method can be used to isolate *P. falciparum* merozoites that remain structurally intact and retain their invasive capacity. Merozoites can then be used in erythrocyte invasion assays, invasion inhibition assays, approaches to image merozoite invasion events by microscopy, evaluating immune responses to merozoites, and other applications.

Introduction

Reagents

VarioMACSTM separation magnet system and columns (Miltenyi Biotec).

18G drawing needle.

RPMI-HEPES standard culture medium containing:

- Albumax II (or heat inactivated human serum)
- glutamine
- hypoxanthine
- gentamicin

U-bottom 96 well tissue culture plate (Falcon 3077)

human erythrocytes

10 mL disposable plastic syringe

1.2µm syringe filter (product code 17593; Sartorius)

PBS

E64 (trans-Epoxysuccinyl-L-leucylamido(4-guanidino)butane, Sigma product number E3132)

Equipment

Procedure

1. Preparation of *P. falciparum* parasites
A) Tightly synchronise (within a 6 hour spread of developmental age) 100-150 ml of parasites at 3-5% parasitemia (2-3% hematocrit)

Recommended synchronisation methods:

- Heparin synchronization allows for tighter synchronization of parasites, within a known window of invasion (Boyle et al., 2010b). This method is better suited for parasite lines that do not form gametocytes and have a close to 48 hour replication cycle. We routinely use D10-PfPHG parasite line (Wilson et al., 2010) that express GFP. D10 parental, 3D7, W2mef and FCR3 wild-type and knockout lines have also been used for isolation of merozoites. Gametocytes from these lines, if a problem, can usually be reduced by inclusion of a regular sorbitol lysis step (once a week or so).
- Repeated or double sorbitol synchronising may also result in cultures of sufficient synchronicity; however, it will be likely that invasion efficiency of isolated merozoite will be reduced.

B) 100 ml of tightly synchronised culture, 3-5% parasitemia, 2-3% haematocrit will typically result in approximately 4 X 10^8 isolated merozoites (Boyle et al., 2010a). This amount is sufficient for approximately 60 aliquots of 50 ml volume samples for invasion inhibition assays.

C) When parasites are at early segmented schizont stage (approximately 40-44 hours post-invasion, characterised by a dappled colouration in a Giemsa stained thin smear) pellet cells by centrifugation (2800 rpm, 5 mins) and resuspend in a reduced volume (20 ml) of culture medium or RPMI-HEPES with sodium bicarbonate and proceed to magnet purification of schizonts.

2. Magnet purification of schizonts and E64 treatment

A) Use the VarioMACSTM separation magnet system and CS columns (Miltenyi Biotec) for magnet purification

- Assemble magnet as per manufacturer's instructions.
- Wash CS magnet column with 50 ml of RPMI-HEPES, ensuring that no air bubbles remain (it is possible to use larger columns for higher volumes of parasite cultures).
- Pass schizonts in reduced volume of medium slowly through column (Eg. 20 ml suspension of schizonts as prepared in step 1c). Typical speed used is approximately 1-2 drops per second through the magnet.
- Wash column while still attached to magnet with 30ml volume of RPMI-HEPES. It is important to wash column extensively to completely remove all uninfected RBCs.
• Wash column with 5-10 ml of culture medium.
• Remove column from magnet and elute parasites with 30-40 ml of culture medium. It may be necessary to remove air bubbles at this stage by gently filling the column from the bottom with the side syringe.

B) Add E64 to eluted parasites at final concentration of 10 mM

• Stock solution of E64 at 10 mM in H2O, filter sterilised, is added at 1/1000 to cultures
• NOTE: If E64 is added to late trophozoite parasite before clear segmentation, treatment will result in trophozoite death which will subsequently block or damage the filter when isolating merozoites, therefore it is important that the majority of the culture has clearly started to segment prior to the addition of E64.
• NOTE: It is important to make clear thin smears to accurately judge lifecycle stage. Typically, this is achieved by pelleting 20-30 mL of culture down in a microcentrifuge tube, removing the supernatant and making a smear of the pellet, then staining using Geimsa or other stains.

C) Return purified schizonts to incubator under normal culture conditions for 5-8 hours.

3. Isolation of merozoites

Prepare parasites for isolation of merozoites

• After 5-8 hours of incubation with E64, smear culture pellets, stain slides, and examine smears to ensure that a large proportion of parasites have formed fully mature sacks of merozoites (ideally >70%; the proportion that are fully matured will be dependent on the synchronicity of the original culture) (refer to Figure S1, (Boyle et al., 2010a) http://www.pnas.org/content/107/32/14378/suppl/DCSupplemental)
• Subsequently, pellet treated schizonts by centrifugation at 1900g for 5 minutes.
• Remove medium and resuspend culture in the required volume for the assay plus at least 500 ml to account for loss during filtration (40 ml of merozoite is needed per assay well for invasion assays, for 60 well assay plate total volume needed is 2400 ml, so filtrate volume of at least 2900 ml is needed). Since the first 200-300 ml of sample is mainly media with few merozoites, it is recommended to filter in a minimum of 1 ml.
• NOTE. It has been reported that Albumax can affect filtration of merozoites through membranes (Blackman, 1994). We filter merozoites using RPMI-HEPES culture media supplemented with 10% human serum or RPMI-HEPES incomplete culture media lacking supplemented protein.
• For isolation of merozoites, prepare the filter by removing the plunger from a 10 ml syringe and attach it to the 1.2 mm filter unit.

• NOTE: Manufacturer’s instructions for 1.2 mm filter indicate that the use of syringe units smaller that 10 ml can result in pressures that may damage the filter unit.

• Add schizonts to syringe, add plunger, and filter merozoites at an even speed into a pipetting tray.

Troubleshooting

Time Taken

Anticipated Results

References


