Guidelines for the Cryopreservation of field isolates

WorldWide Antimalarial Resistance Network (WWARN)



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# 1. Purpose

The purpose of this document is to standardize the methodology for cryopreservation of malaria parasites using glycerolyte. This should be considered as a recommendation or as a definition of minimum requirements, created by the Worldwide Antimalarial Resistance Network (WWARN), aiming to achieve homogeneous quality in the pre-analytical phase of studies and to provide guidelines in the processes of collection, processing, preserving, storage, shipment and safe handling of samples from human origin.

# 2. Scope

This document applies to those sites wishing to collect clinical isolates or specimens for *in vitro* antimalarial drug susceptibility assays.

**NOTE: It is recommended that the analytical laboratory where the *in vitro* assays will be done is contacted before commencement of the study to inquire about specific requirements of that particular laboratory.**

# 3. Abbreviations

WWARN Worldwide Antimalarial Resistance Network

ARC Asia Regional Center

PBS Phosphate buffered saline

# 4. Duties and Responsibilities

The tasks to be completed for this procedure are listed below. Each of these must be assigned to an individual(s) who has been trained to perform these tasks and in the use of relevant health and safety precautions.

* Proper identification of patient and matching samples
* Collection of samples
* Processing of samples

# 5. Materials

* Samples for cryopreservation of malaria parasites must be taken into sterile heparinized plastic tubes and transferred to screw cap polypropylene cryovials for transportation and storage. Use of plastic sampling and storage containers improves safety at the site.
* *Glycerolyte® (Fenwal Co. Ltd, Canada)*
* RPMI-1640 or phosphate buffered saline (PBS) or normal saline to wash packed red cells.
* 50 mL sterile conical tubes.

# 6. Procedure

* *6.1 Blood samples*
Blood samples should be taken into a sterile heparinized tube. Blood containing *P.* *falciparum* infected red cells with at least 0.5 % parasitaemia is suitable for cryopreservation.
* *6.2 Centrifugation*Samples are centrifuged at 800 – 1000 ×*g* for 5 minutes and then plasma and buffy coat are removed. The packed red cells are washed with RPMI-1640 (or PBS or normal saline) by centrifugation at 800 – 1000 ×*g* for 5 minutes for a total of three times. After the supernatant has been discarded, the cell pellet should be transferred to a 50 mL sterile conical tube.
* 6.3 *Freezing samples with glycerolyte® (Fenwal Co. Ltd, Canada)*
1. Measure the volume of packed red blood cells
2. Calculate the first volume of glycerolyte to be added into the packed red cells:

If volume of packed red cells = V, then the first volume of glycerolyte to be added into the pack red cells = 0.33 × V

e.g. Volume of packed red cells

= 1.5 mL

The first volume of glycerolyte to be added into the packed red cells

= 0.33 × 1.5

= 0.5 mL

1. Add the first volume of glycerolyte into the packed red cells very slowly
adding one drop at a time with very **gentle and continuous mixing.**

*Note*: This step should take at least 3 – 5 minutes.

1. Stand for 5 min at room temperature to allow the glycerolyte to permeate the cells.
2. Calculate the second volume of glycerolyte to be added into the cell
suspension (from iii.):

If volume of packed red cells = V, then the second volume of glycerolyte to be added into the packed red cells = 1.33 × V

e.g. Volume of packed red cells

= 1.5 mL

The second volume of glycerolyte to be added into the packed red cells

= 1.33 × 1.5

= 2.0 mL

1. Add the second volume of glycerolyte into the packed red cells by adding the glycerolyte still one drop at a time with **gentle and continuous mixing.**

*Note*: This step should also take at least 3 – 5 minutes.

1. Transfer the cell suspension into a cryotube.
2. Keep at –80 ºC **overnight** thentransfer to liquid N2 for longer term storage.

# 7. Remarks

1. Blood samples containing *P.* *falciparum* infected red cells with 0.5 % parasitaemia are the most suitable for cryopreservation, though samples with lower parasite densities may be acceptable. This should be confirmed with the *in vitro* analytical laboratory.
2. All centrifugation/washing steps should be performed at 4 ºC in a refrigerated centrifuge if one is available.
3. This procedure is best performed in a biosafety cabinet but it is not required. The procedure can also be performed in a clean lab area, protected from air drafts, in order to reduce as much as possible the exposure of the specimen to contaminants.
4. If a –80o C freezer is not available, a –20o C freezer can be used. If no freezers are available, keep the packed cell suspension in glycerolyte at 4o C for 1 hour before transferring to liquid nitrogen.