Leukocyte Depletion of Whole Blood Using CF11 Columns v1.3

Procedure

Molecular Module

WorldWide Antimalarial Resistance Network (WWARN)



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Version History

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| Version number | Revision(s) & reason for amendment | Release date |
| 1.3 | Updates to procedure | 18/05/2016 |
| 1.2 | Updates to procedure: column storage (6.1.IV) and pre-column centrifugation (6.3) | 18/03/2011 |
| 1.1 | Changes to template | 27/10/2010 |
| 1.0 | Creation of procedure | 27/07/2010 |

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# 1. Purpose

This procedure is designed to reduce the amount of human DNA in *Plasmodium*-infected whole blood samples for genomic and transcriptional studies by removing human leukocytes (white blood cells) using cellulose columns.

# 2. Scope

This procedure is intended for use in studies where whole blood samples are collected for next-generation sequencing, genotyping and transcriptional analysis of *Plasmodium* infections. It describes, storage and use of cellulose columns for removal of white blood cells from whole blood at the time of sample collection. It also describes methods for storing blood samples for DNA and RNA isolation after leukocyte depletion.

# 3. Abbreviations

EDTA Ethylene-diamine tetra-acetic acid

WBC White Blood Cell

PBS Phosphate Buffered Saline

PPE Personal protective equipment

# 4. Duties and Responsibilities

N/A

# 5. Materials and Equipment

5.1 Materials

1. Blood collection for parasite DNA

* 5-10 mL EDTA vacutainers

1. Cellulose column assembly and storage

* Cellulose powder (Advantec Cellulose Type B, catalogue No. 49020020 OR Macherey-Nagel Cellulose, catalogue no. MN 2100 ff)
* Whatman Grade 105 lens cleaning tissue (100 × 150 mm, 25 wallets of 25 sheets, 25/pack  Whatman catalogue no. 2105-841)
* 10 mL plastic syringes, centred, not offset (such as 100 pack, Becton-Dickinson catalogue no. 309604)
* Desiccant
* Zip-locking bags

1. Cellulose column filtration

* Cellulose columns
* Isotonic PBS (pH 7.4, without Mg2+ and Ca2+)
* 15 mL and 50 mL centrifuge tubes
* Serological pipets (5 or 10 mL) and/or plastic transfer pipets (5 mL)
* Sticky tape

1. Storage of packed red blood cells for DNA & RNA isolation

* Trizol reagent (Invitrogen)
* Micropipette tips or transfer pipets
* Internal thread screw cap cryovials for shipping and storage

5.2 Equipment

* Benchtop centrifuge for 15 mL and 50 mL tubes (refrigerated centrifuge optional)
* Forceps, 8 to 10 inch length and able to fit into the barrel of a 10 mL syringe
* Spatula
* Scissors
* PPE for column assembly and Trizol step (use of fume hood optional for column assembly and handling of Trizol)
* 200 µL or 1000 µL micropipette (optional)
* Pipet controller for 5/10 mL serological pipets (optional)

# 6. Procedure

6.1 Cellulose column storage

The cellulose powder and columns used in this procedure are sensitive to humidity and must be stored and used properly in the field. Please follow the instructions below to ensure the best results at your site.

* Store the powder and prepared columns in air-tight containers; columns should be stored with desiccant. Exposure of the columns to air should be avoided. Do not repeatedly open and close the containers.
* If desiccant begins to change colour, it should be replaced with fresh desiccant.
* Cellulose powder which has been exposed to humidity looks ‘sticky’, with clumps of white cellulose powder that are not saturated by the sample when used. These columns will also take longer for the sample to flow through. If you notice this, the cellulose in the columns should be ‘loosened’ by retracting the plunger (but not removing it from the syringe), tapping the syringe to loosen the cellulose until no clumps are visible, and re-compacting the cellulose to 7 mL.

6.2 Blood collection

* Collect at least 5 mL of venous blood on EDTA. Up to 10 mL of blood can be collected from adults.
* A minimum parasite density of 5000/µL is recommended but this procedure is likely to work best with parasite densities of ≥ 10000/µL.
* Samples should be processed immediately or stored at 4 °C until they can be processed. Leukocyte depletion must be completed preferably within 6 hours but up to 24 hours may be acceptable as well.

**NOTE**: Leaving the blood at room temperature or freezing the blood at this stage will result in haemolysis and significant sample loss.

6.3 Procedure for leukocyte depletion

One column should be used for **up to 5 mL blood**. 10 mL blood samples should be split between two columns.

* Compress the cellulose column to 7 mL if needed and remove the plunger from the syringe. If cellulose powder is displaced by air while removing the plunger, push it back in to secure column and gently remove. Suspend over an uncapped 50 mL tube using a clamp or tape.
* Apply 6 mL of PBS to the top of the column (barrel of syringe).
* Transfer blood to a 15 mL centrifuge tube in 5 mL aliquots to match the number of columns used. For a 10 mL blood sample, transfer 5 mL blood to each of two 15 mL tubes. Centrifuge at 1000 × g for 5 – 10 minutes (at 4 ºC if a refrigerated centrifuge is available), until there is a clear separation of the red blood cell pellet from supernatant plasma.
* Carefully remove and discard as much of the plasma as possible. A small amount of the buffy coat may also be removed.
* Add PBS to the blood so that the total volume equals twice the original blood volume and swirl or gently pipet up and down to mix.
* Once the PBS applied previously is no longer visible at the top of the column, apply blood-PBS mixture to the top of the column. Each tube of blood-PBS mixture is added to a separate column. Allow sample to pass through by gravity. Depending on volume you may need to apply the sample in two steps to prevent overflow in the syringe.
* Once fluid is no longer visible at the top of the column, add 6 mL PBS to the top of the column and allow it to pass through. If necessary, carefully re-insert the plunger and gently push to pass the remaining sample through the column in a drop-wise fashion.

**NOTE:** Once the plunger has been placed into the column, additional PBS cannot be added as removal of the plunger will cause air to disrupt the column.

* Centrifuge the filtered sample at maximum speed at room temperature for 5 – 10 minutes, until the red blood cells pellet and there is a clear separation of pellet from supernatant.

**NOTE:** If there is a ‘brake’ function on the centrifuge, it should be turned off. Braking at the end of the centrifugation may cause the red cells to be resuspended in the buffer.

* Carefully discard supernatant, taking care to not disrupt the packed red blood cell pellet.

6.4 Storage of leukocyte-depleted packed red blood cells for DNA and RNA

* Gently pipet leukocyte-depleted packed red blood cell pellet up and down to mix.
* Transfer up to 500 µL packed red blood cells to a 5 mL internal thread screwcap cryovial labelled with study subject number, initials, and date and time of sample collection and sample type.
* Add 4 mL Trizol reagent to the tube and mix well by gentle inversion.

**NOTE:** Trizol reagent contains phenol chloroform. Be sure to use PPE when using Trizol. Avoid inhalation and contact with skin. Use in a well-ventilated area or under a fume hood if available. For preservation of RNA, 5-10 volumes of Trizol must be added to 1 volume of specimen. If more or less than 500 µL of packed red cells are processed for RNA preservation, the volume of Trizol must be adjusted accordingly.

* The remainder of the cell pellet is to be stored for DNA extraction. Transfer remaining leukocyte-depleted packed red blood cells to an internal thread screwcap cryovial labelled with study subject number, study subject initials and date and time of sample collection, and sample type.
* Store both tubes in a –80 ºC freezer or in liquid nitrogen. Pellets should remain frozen until the time of DNA and RNA isolation.

# 7. References

WorldWide Antimalarial Resistance Network (WWARN) Molecular Module. 2011. Leukocyte Depletion of Whole Blood Using CF11 Columns v1.2. WWARN Procedure.

Sriprawat K, Kaewpongsri S, Suwanarusk R, Leimanis ML, Lek-Uthai U, Phyo AP, Snounou G, Russell B, Renia L, Nosten F. Effective and cheap removal of leukocytes and platelets from *Plasmodium vivax* infected blood.*Malaria Journal* 2009; 8:115.

# Appendix: Conversion between RCF (x g) and RPM

Relative centrifugal force, or RCF (measured in times gravity or × *g*), depends on both revolutions per minute (RPM) and the radius of the centrifuge’s rotor. The relationship between RCF (x *g*) and RPM is as follows:

*g* = (1.118 × 10-5) \* (radius of rotor in cm) \* (RPM)2

A conversion calculator can be found at:

<http://www.beckmancoulter.com/resourcecenter/labresources/centrifuges/rotorcalc.asp>

A conversion table can be found at:

<http://www.piercenet.com/files/TR0040-Centrifuge-speed.pdf>