Institute of Health Carlos III

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SOP
Erythrocytes

Blood Products Working Group

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Collection, Processing and Storage of Erythrocyte Samples

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1. ABBREVIATIONS

- **BHT:** Butylated hydroxytoluene
- **PBS:** Phosphate buffered saline
- **EDTA:** Ethylenediaminetetraacetic acid. EDTA attracts calcium ions, thus blocking the coagulation cascade (dipotassium salt K2 or K3).
- **ACD:** Citric acid, citrate and dextrose in amounts of 0.9, 2, and 2 g, respectively, in 120 ml of distilled water. It is used to obtain plasma for coagulation and platelet function assays. It is used in collection and storage for transfusion since it preserves the blood longer, particularly the survival of erythrocytes: 21-32 days-70% survival. It changes the calcium concentration.

2. DEFINITIONS

- **Freezing solution:** Composition: 28% (v/v) or 35% (w/v) glycerol, 3% Mannitol, 0.65% NaCl, ddH2O.
- **Erythrocytes:** are the most numerous elements of the blood; their function is to transport oxygen to the various tissues of the body. The amount considered normal fluctuates between 4,500,000 (in women) and 5,000,000 (in men) per cubic millimeter (or microliter).

3. OBJECTIVE

The objective of this procedure is to define the course of action and to establish the basic quality guidelines with respect to collecting and handling and to the processing of erythrocyte samples that will be deposited in biobanks belonging to any center or hospital affiliated to the National Biobank Network.

4. SCOPE

This procedure applies to all erythrocyte samples that are obtained in order to be stored in a biobank. This protocol does not detail the occupational health and safety processes regarding biohazardous materials and/or chemical products, and it is recommended that the personnel follow the Health and Safety rules established in each center.

5. MATERIALS AND SERVICES

- Courier Service holding a permit for the transport of biological materials:

Material	*UN Classification*		*Packing instructions*				*Comments*
	Class	*No.*	*ADR*	*RID*	*ICAO*	*IMDG*	
Infectious samples affecting humans	*6.2*	*2814*	*620*	*620*	*692*	*620*	*Materials groups 2, 3, 4*
Diagnostic specimens	*6.2*	*3373*	*650*	*650*	*650*	----	*Materials groups 1, 2, 3*

- For non-infectious samples: Bag or container for internal transport in the hospital.
- For infectious or hazardous samples: Transport container for dangerous substances that complies with the effective legislation: Royal Decree 664/97, following "Packing Instruction 620 (IATA - ICAO 602)"
  - Syringes and/or material required for collecting blood
  - Gloves
  - 15mL ml PP tube
  - Sterile 3.5 mL transfer pipettes
  - 2 ml cryotubes
  - 1X PBS
  - 1 mM BHT (butylated hydroxytoluene)
  - Freezing solution
  - Automatic micropipette (volume 0.5-10 µL)
  - Sterile tips for micropipette
  - Centrifuge
  - Analytical balance

6. DEVELOPMENT

6.1. COORDINATION FOR THE CORRECT COLLECTION AND RECEIPT OF BLOOD SAMPLES IN THE BIOBANK

6.1.1. Blood must be collected after the patient has signed the informed consent for donating samples to the biobank.

6.1.2. Blood is collected via peripheral venipuncture. The people in charge of carrying out this procedure and of programming extractions must coordinate with biobank staff to ensure that the blood collection tubes with anticoagulants are properly identified and that a proper collection and receipt of the sample is guaranteed. The type of anticoagulant used must be the most appropriate one for studies for which the sample is intended. Based on previous experience, the following is advised:

a) K2 or K3 EDTA if other types of products will be extracted from the sample, such as plasma or serum.

b) Heparin if the sample is also intended for cell proliferation studies.

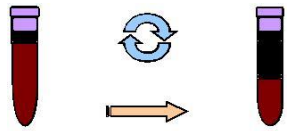
c) ACD if only erythrocytes are to be extracted.

6.1.3. It is advised to take the maximum possible information concerning the sample at the time of extraction:

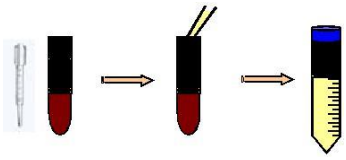
- Date and time of extraction.
- Type of anticoagulant.
- Incidents not related to the protocol.

6.2. SEPARATION OF ERYTHROCYTES FROM WHOLE BLOOD

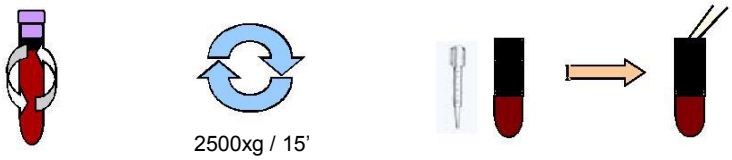
6.2.1. Centrifuge the tube at 2500xg for 15 minutes at room temperature.



6.2.2. Aspirate the plasma with a transfer pipette, avoiding aspiration of leukocytes (they form a thin white layer above the red cells), and transfer to a 15 ml tube. If the plasma needs to be stored process it immediately following the corresponding SOP.

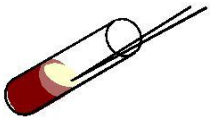


6.2.3. Fill the tube with 1X PBS at room temperature and mix by inverting. Centrifuge at 2500xg for 15 minutes at room temperature. Discard the supernatant, avoiding aspiration of leukocytes (they form a thin white layer above the erythrocytes).



6.2.4. Repeat wash with 1X PBS

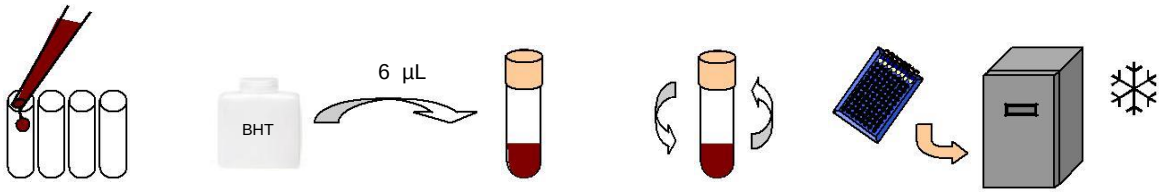
6.2.5. Aspirate the white leukocyte layer with a transfer pipette together with part of the erythrocytes and transfer to a 15 mL tube. The aspirated volume must be 1.5 mL and corresponds to a red pellet. Process leukocytes if required.



6.3. PROCESSING ERYTHROCYTES FOR THE DETERMINATION OF MEMBRANE LIPIDS

6.3.1. Pipet 600 µL aliquots of erythrocytes into properly labeled 2 mL microtubes, and add 6 µL of 1 mM BHT to each aliquot. Mix gently by inversion.

6.3.2. Freeze at -80ºC



6.4. PROCESSING OF THE SAMPLE TO PRESERVE ERYTHROCYTE INTEGRITY

6.4.1. Weigh the remaining erythrocyte package and add a volume of Freezing Solution of the same weight as the package.

6.4.2. Allow to stabilize for 20 minutes at room temperature (22ºC).

6.4.3. Freeze by immersion in liquid N2 and store frozen in liquid N2 or in N2 vapor.

6.5. MAINTAINING TRACEABILITY AND DATA ASSOCIATED TO THE SAMPLE

Biobanks advise to gather the maximum amount of information possible concerning the sample, both at the time of reception and after processing and storage, and depending on the studies for which they will be used, for example:

- Date and time of receipt and/or processing
- Degree of hemolysis
- Volume of blood received
- Degree of Lipemia
- Degree of Jaundice
- Degree of coagulation
- Incidents during processing

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