



Manual of Procedures FLADEX project

MOP Chapter 6: Blood samples



Promoting Fitness & Health
through Physical Activity



UNIVERSIDAD
DE GRANADA



Chapter 6. Blood samples

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1. Background

The FLADeX project aims to gather fasting blood samples during three specific conditions: Condition A (Aerobic exercise), Condition B (Resistance exercise), and Condition C (Control condition), utilizing the common venipuncture procedure. In every condition, there will be four different blood withdrawals (PRE, minute 0, 50 and 70 after condition). Following the study's aims, we plan to conduct various assays on AD blood-based analytes to explore potential mechanisms through which exercise enhances brain health in older adults. Additionally, surplus blood will be stored for prospective ancillary studies and assays.

The goal of the flADex Blood Sample Collection and Processing MOP chapter is to ensure the accurate collection, processing, shipping, and central storage of blood specimens. Adequate handling is crucial, as any errors in collection, processing, or storage may compromise the integrity of completed assays. Blood collection involves drawing approximately 50 ml at each of the three specified conditions (around 12mL per blood draw).

All blood draws will be performed by an expert nurse staff member, and subsequent processing will be conducted by research team members with requisite laboratory and bloodborne pathogen training.

2. Equipment list

2.1. Blood draw equipment list

- Alcohol wipes
- 21-gauge needles
- BD vacutainer Luer-Lock
- Butterfly needles
- Disposable gloves
- Paper towels
- Disinfectant cleaner (Kills HIV and HBV)
- Gauze pads
- Tourniquets
- Band-Aids
- Blood collection tubes (EDTA K3 Purple (4mL) and Yellow Serum (3.5mL))



Figure 1. Blood tubes needed for the blood withdrawal of FLADeX project.



2.2. Aliquote equipment list

- Biohazard waste containers
- Tube racks
- 200 y 1000 uL Pipetes
- Tips of 200 uL
- Tips of 1000 uL
- Wilmut eppendorfs (0.65mL)
- Wilmut boxes (12x8)
- Centrifuge
- Ice for sample transport and shipping boxes

3. Blood tube and Wilmut ID labeling

Wilmut labels will be the following. Each tube should be labeled appropriately with the participant ID (same as AGUEDA), Condition (A for Conditon A (Aerobic exercise), B for Conditon B (Resistance exercise) or C for Conditon C (Control condition)), Blood withdrawal timepoint (T0 for baseline, T1 for minute 0, T2 for minute 50 and T3 for minute 70) and the component of the blood extracted (for whole blood W, for plasma P, for leukocytes L and for serum Y). For example, **111_A_T0_P1**, it means that it is the participant nº111 Aerobic Condition, time point for minute 0 and the component of the blood is plasma from purple tube.

For the pilot study the labeling was similar but only changing the blood withdrawal timepoint ((P for baseline, 0 for minute 0, 1 for minute 50 and 2 for minute 70).

4. Test Day procedure

The nurse in charge of blood extractions will have a designated room at the **Research Center for Mind, Brain, and Behavior (lab 11)**, and some considerations will be followed:

- Before the first extraction, the nurse should have 12 blood tubes ready for each participant (8 purple and 4 yellow), along with the necessary materials (2 purple (for whole blood, plasma and leukocytes and 1 yellow (for serum) per blood draw). The line will be inserted in the first blood withdrawal and removed at the end of the morning.
- We must take into consideration that the nurse will need a few minutes to remove the line. After every blood withdrawal, serum will be used to clean the line.
- After that, the instrument **Blood draw timepoint** must be filled by the evaluator. For the next blood withdrawal, the nurse will remove 5mL approximately and then will fill the blood tubes.
- The total blood collected at each time point during the evaluation day will be approximately **12 ml**, amounting to a total of **50 mL** by the end of the day.
- It is **MANDATORY** that the first tube extracted is the serum one (yellow) to avoid contamination.



- The evaluator must complete ***Annex 6.1 Blood Sample Management FLADEX***.
- The vacutainers will be processed at the **lab 11** and the samples will be stored in the iMUDS freezer at -80°C.

5. Transportation and blood processing

Pack the samples in leak-proof containers adhering to all safety regulations helped by employing cool packs while waiting for processing. The processing will start after a participant have completed his/her condition. We should pay particular attention to temperature control requirements. We must acknowledge the time of sample collection and dispatch. Upon arrival at the destination, follow the processing instructions to storage them in the iMUDS freezer.

5.1. Centrifuge

The centrifuge will be set at 3000 RPM at room temperature, with a total centrifugation time of 10 minutes for the centrifugation of ALL the vacutainers. Yellow vacutainers need at least 40 minutes before centrifugation. This will be done in **Lab 4**.

5.2. Pipetting

The purple tubes (plasma) and yellow tubes (serum) should be aliquoted in Wilmut. Include future information related to lab details and characteristics. This will be done in **Lab 11**.

5.3. Storage

Wilmut will be stored in the freezer of iMUDS. Each labeled Wilmut has a designated location within the freezer in a specific box. To determine the corresponding box based on its label, refer to ***Annex 6.1 Blood Sample Management*** for the box's location in the freezer. For each participant in the FLADEX project there will be 3 boxes, inside every box will be the following items:

1. **1 PB (purple blood) + 5 YS (yellow serum) +1 PL (purple leukocytes) + 10 PP (purple plasma) wilmut PER BLOOD WITHDRAWAL. In total 68 WILMUT per Condition.**
2. **Participants will have to get through 3 different conditions, so a total of 3 boxes will be stored per participant into the freezer.**

5.4. Excel data registration.

For every blood withdrawal timepoint, start and finish of it will be recorded in RedCap and afterwards filled in the excel data sheet (***Annex 6.1 Blood Sample Management FLADEX***). Along with it, centrifugation will be also registered.

This process will be done after all the samples were stored in the freezers located at iMUDS. Storage time and position will be recorded as well as any further comments from the sample (hemolyzed, partial...).



This should be done maximum after each day of evaluations and the registration will be in charge of the blood team (Marcos Olvera, Emilio J Barranco).

6. Annex

- **Annex 6.1. Blood Sample Management.**
- **Annex 6.2. RedCap data forms.**
- **Annex 6.3. Instructions for conditions.**
- **Annex 6.4. Breakfast.**
- **Annex 6.5. Label_printing.**