Standardized Operating Procedure

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1. Introduction

This document is a comprehensive data collecting tool which was created to assist all types of researchers. It consists of general guidelines for measuring anthropometric and physiological factors, and blood sampling with reference to the methods mentioned in the literature and the manuals of the equipment.

This document describes the standard operating procedure (**SOP**) for the measurements of anthropometric and physiological factors that include wight, body composition, height, peak power output, heart rate, $VO_{2\ MAX}$, and exhaustion time, and the SOP for blood sampling.

Furthermore, it indicates the **SOP** for measuring plasma hemoglobin and hematocrit, creatine kinase (**CK**) levels, and the cytokines of proinflammatory **IL-6**, and **TNF-α**, and the anti-inflammatory **IL-10**. CK to be measured using an autoanalyzer **(Cobas C111)**, IL-6 **(Cobas C411),** while TNF-α and IL-10 with ELISA.

The research assistance team are fully responsible for following the guidelines which should be governed by the ethics code.

2. General guidelines

Before starting the experiment, you need to be familiar with the tasks you are planning to do, the equipment you are responsible for, and the machines you are going to use. Below are some of the main guidelines:

- Use the equipment according to its manual.
- Clean the tools and equipment after each use.
- Sample labeling, handling and storage conditions are crucial and affect the success of your experiment.
- Create an identification code for each participant for confidentiality purposes.
- Measurements and data recordings must be taken in a pair.
- Follow safety rules in the laboratory.

Anthropometric Measurements

- Height measurement guidelines

You will measure the weight of the participants using digital column scale (**Seca GmbH**) according to the following steps:

1. Calibrate the scale and place it on a flat surface.
2. Instruct the participant to take off shoes and any hair attachments and stand straight against the backboard of the scale with shoulder and head touching the wall.
3. Participants' heels should be close to each other.
4. Record the measurement once displayed.

- Guidelines for measuring weight and body composition

Weight and body composition will be measured using the same device **(InBody 720, Precise Body Composition Analyzer).** Below are the steps to be followed:

1. Advice all participants to:
   - Refrain from food or drinks 3 hours before taking the measurement.
   - Wear light clothes.
   - Empty bladder at least 30 minutes prior to testing.
2. Ask the participant to take off shoes and to get rid of any heavy item that would affect the reading.
3. Participant should stand barefoot on the metal foot electrodes with holding the hand electrodes firmly and straight down.
4. Instruct the participant to keep calm with no movement until you take the reading.
5. Test repeats should be taken at the same time of the day under similar conditions.

Figure 1 InBody 720 body composition analyzer (1)

- Determination of peak power output

With the help of a cycle-Ergometer, we can check the determine both peak power output (PPO).

Cycle-Ergometer guidelines

1. It is a machine with a manual you need to follow to finish the setup.
2. Ensure the participant wears proper exercise clothing.
3. Connect all sensors and place them on the participant's body.

4. Under full supervision, instruct the participants to warm up their muscles at low intensity.
5. Start increasing the resistance following the predetermined protocol.

Peak power output (PPO)

When the participant is no longer able to continue pedaling, a cool down at low intensity should be followed, and the PPO is the highest level of power in (watts) is recorded.

- **Measuring Heart rate guidelines** [2]

1. Connect the Polar H10 heart rate sensor before the start of the intended physical activity.
2. Place it below the chest muscle, and don't make it too tight nor too loose.
3. The electrodes should be moistened with conductive gel or water.
4. Pair it with a compatible device (mobile, tablet or any fitness equipment) using Bluetooth.
5. After the finish of the activity, wait a few minutes to ensure the device records the heart rate during the recovery period.
6. Disconnect the strap and rinse it with water if necessary.

- **The guidelines for the assessment of the maximal oxygen consumption ($VO_{2\ MAX}$)** [3]

1. Place the necessary sensors and electrodes on the participant, which may include a face and a pulse oximeter for monitoring oxygen saturation prior to the start of the training.
2. Calibrate the metabolic cart to room temperature (21 – 23 °C) and to gases of the concentration (16.00% O_2 and 3.99% CO_2)
3. VO_2 is recorded every 15 seconds during the exercise as, and when it reaches a plateau, the $VO_{2\ MAX}$ is achieved.
4. $VO_{2\ MAX}$ is expressed in mL of oxygen / kg of body weight (mL/Kg/min).

Blood Sampling

- **Taking blood sample**

It is necessary to follow strict guidelines when taking a blood sample from the participant/ patient, and it should be done by a licensed technician. Below are the steps:

1. Prepare all the supplies to be used.

2. Wash your hands and wear clean gloves.
3. Apply a tourniquet a few centimeters above the puncture site and identify a suitable vein.
4. With alcohol swab, clean the area and allow it to dry.
5. Insert the needle into the vein smoothly and connect it to an anticoagulant tube to allow the blood to flow into it.
6. Disconnect the tube, and then remove the needle.

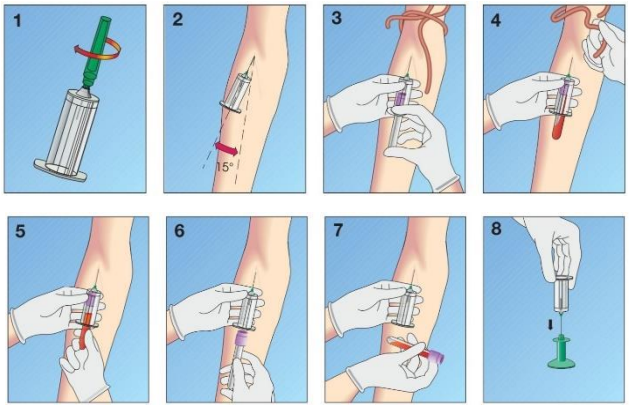

Figure 2 Steps for taking blood sample [4]

7. Apply a cotton ball to the puncture site to stop the bleeding, and then apply a bandage over the puncture site.
8. Discard the needle in a designated medical waste container.
9. Label the tube properly.
10. Invert the tube gently to mix the blood.
11. Store it at (4 – 8) °C for a maximum of 24 hours.

- **Collecting blood plasma**

From the blood sample collected from the participants, plasma can be collected as follows:

1. Centrifuge the blood sample at (3200 to 3500) rpm for 10 to 15 minutes.
2. Transfer the top plasma layer to another tube.
3. Store at -80 °C for further analysis.

Hemoglobin and Hematocrit measurement

The laboratory device **HemoCue** is to be used to measure both hemoglobin and hematocrit levels in the blood. Below are the steps [5]:

1. The cuvette holder of the device should be in the loading position.
2. Ensure the participant's hand is warm and relaxed.
3. Clean the participant's ring or middle finger with disinfectant and let it dry.
4. Stimulate blood flow by applying a light pressure from the top of the knuckle toward the fingertip.

5. Use a lancet to prick the finger.
6. Wipe away the first (1-3) blood drops.
7. Press again lightly until another drop appears and transfer it onto the microcuvette of the HemoCue.
8. Wipe off any excess blood from the edges of the microcuvette.
9. Insert it into the HemoCue device.
10. Push the holder to its measuring position.
11. The hemoglobin and /or hematocrit concentration is displayed on screen.
12. Discard the microcuvette after measurement.

Creatine kinase (CK) assay

CK can be quantitatively determined from blood plasma sample using cobas c111 bench-top analyzer from Roche Diagnostics.

- **Guidelines for using c111 analyzer to measure CK** [6]

1. Switch on the machine.
2. Log on the system
3. Check the internal fluids including wash bottle that is filled with distilled water, in addition to the liquid and the solid waste containers.
4. Load the reagents from the CK kit, and the other materials required for the test in the reagent disk in the analyzer.

- Kit reagents are: R1(Imidazole buffer) and SR (CAPSO buffer)
- The other materials: Calibrator f.a.s., Precinorm U plus, Precipath U plus, Preci Control Clin Chem Multi 1, Preci Control Clin Chem Multi 2, and NaCl Diluent 9 %.

5. Prepare the system from the screen.
    a. Perform a maintenance action.
    b. Scan the reagents.
    c. Check the cuvettes.
    d. Perform mixing.
    e. Identify the sample.
6. Perform calibrations.

7. Perform quality control, if pass, you can start the test.
8. Identify the sample.
9. Select the tests.
10. Place your sample tubes (up to 8 samples per run)
11. Start the run.
12. The cobas c 111 analyzer automatically calculates the analyte activity of each sample. Conversion factor: U/L x 0.0167 = µkat/L

Cytokines measurement

The proinflammatory cytokines Interleukin-6 (IL-6) can be determined using Cobas analyzer e411, while the pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNF-α) and the anti-inflammatory cytokines Interleukin-10 (IL-10) can be determined with ELISA.

- **Guidelines for using cobas analyzer e411 to measure IL-6** [7]

1. Prepare the machine.
   a. Make sure the surfaces of the analyzer and the probes are clean and undamaged.
   b. Check the pinch valve tubing.
   c. Ensure the pipetter syringes and tubing have no bubbles.
   d. Fill the wash bottle with distilled water and SysWash.
   e. Empty the liquid waste bottle and waste box. (if necessary)
2. Switch on the machine
3. Bring the cooled reagents to 20 °C and place them in the reagent disk of the analyzer (its temperature 20 °C)
4. From the screen, start a calibration. Calibration must be performed once per reagent lot using fresh reagent.
5. Perform a quality control check.
6. Centrifuge the sample tubes (if necessary) and then place them in the analyzer (up to 30 samples/run), and note that samples should be cooled to 20 °C.
7. Program the samples and select the test.
8. Run the test.
9. Remove samples from the analyzer after the test finishes.

10. After the test finishes, the analyzer automatically calculates the analyte concentration of each sample in pg/mL (picograms per milliliter).

- **Guidelines for ELISA** [8]

1. Prepare all reagents, samples and standards as instructed in the kit.
2. Add 50 µL of your sample or standard to appropriate wells.
3. Add 50 µL of the antibody cocktail to each well.
4. Seal and incubate for 1 hour at room temperature on plate shaker set 400 rpm.
5. Aspirate and wash each well with 3 x 350 µL 1x wash buffer for at least 10 seconds.
6. After the last wash, invert the plate on a clean paper towel.
7. Add 100 µL of TMB development solution and incubate for 10 minutes at 400 rpm plate shaker.
8. Add 100 µL stop solution to each well followed by 1 min shake.
9. Record at 450 nm.

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