# 015: Sampling and conditioning for cryostorage

Several samples and medical data were collected during the visit of the conscripts at the laboratory, as follows:

1. **Identification of the volunteers:** contacts with the volunteers was made by the medical supervisor of the study. He established weekly the list of conscripts who accepted to participate in the biological part of the study and sent it to the laboratory in charge of collecting the samples.
2. **Identification labels:** a set of labels was printed for each volunteer with the name and study identification code, date of sampling, lab name. The identification number refers to the unique number given to the conscripts as they entered into the study. The date refers to the date of collection.
3. **Medical examination**: the medical doctor received the list with the names and the codes. The doctor performed the clinical examination of the genitals and collected several parameters (see urological examination). The medical doctor provided the conscripts with all labelled recipients needed for the samples (blood, urine, sperm).
4. **Blood sampling**: the medical doctor or a trained lab personal collected 2 tubes containing 10 mL native blood. These tubes were left at ambient temperature for 30-60 min to allow for coagulation. The tubes were then centrifuged (4000 g, 10 min). The serum was carefully removed and deposited in two 5mL labelled plastic tubes.
5. **Urine collection**: the conscripts were given a urine sampling device to collect up to 50 mL urine. This was performed prior to sperm collection. The urine collected was distributed in 2 tubes of 15 mL, securely closed with a screw cap. These tubes were labelled with the Identification number and the date.
6. **Sperm collection**: the sperm was collected by masturbation in previously weighed collection devices consisting of a funnel attached to a 15 mL tube. After collection, the devices were weighed again. Any loss of sperm liquid during ejaculation was documented. The sperm volume was obtained by the difference in weight. The sperm was left for 30-40 min on a heating plate (37°C) until fully liquefied. The necessary amount of sperm was used for concentration and motility (up to 20 µL). Two smears were prepared using 5µL for each one. The sperm left over was then centrifuged at 2000 TPM for 10 min.The supernatant corresponding to the seminal fluid was transferred into a 5mL tube. The pellet was transferred also into a 1mL

7. At the end of the collection period, the following samples were obtained and verified:
    a. A urology questionnaire filled out
    b. 2 tubes of urine, to be stored in a box URINE
    c. 2 tubes of serum, to be stored in a box SERUM
    d. 1 tube of blood clot, corresponding to the centrifugation pellet, to be stored in box BLOOD
    e. 1 tube of seminal fluid, to be stored in box SEMINAL FLUID
    f. 1 tube of sperm pellet, to be stored in box SPERM
    g. 2 slides for morphology
8. All freezing boxes were stored in a freezer at -20°C, for up to 4 months. They were then sent in stiropor packages mixed with dry ice, either by mail or by car to the FABER Foundation in Lausanne. Upon arrival the boxes were checked and stored in -80°C freezers.
9. At the end of the study, all freezers used in FABER were dispatched to the CMU UNIGE by truck, while keeping the freezers under control. Upon arrival, the freezers were reconnected to the power supply and all boxes were individually checked.
10. At the CMU UNIGE freezing facility, freezers are under continuous monitoring for temperature (-80°C). A centralized unit supervises all connected freezers and in case of sudden loss of freezing capacity a series of calls are made to all declared responsibles, until one of them is reached. In case of no response, a transfer of all material to a special freezing unit is automatically performed by the technical staff (24h/7).

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