

## Online Supplemental file 5: Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies

In the CV-PREVITAL Study, each IRCCS recruits a number of participants to whom, in addition to the questionnaires, samples of biological material (blood, saliva or feces) are taken. The biological material collected is stored in the cryospaces dedicated by each IRCCS to the Widespread Biobank of the Italian Cardiology Network. In order to harmonize the collection and storage of samples, Standard Operating Procedures (SOPs) has been created for the Widespread Biobank and shared among the participating centers for the management of the sample from the patient recruitment and signing phase of the informed consent, to the collection and storage of biological samples and possible redistribution of the aliquots. The harmonization of sample collection also includes the use of the same container for sample collection, the same cryovials for storage, and the same codes for pseudonymization of samples. All aliquots are stored in cryotubes with QR Code to facilitate the distribution and sharing of samples among the recruiting centers of the CV-PREVITAL study or with other national or international institutes.

### SOPs for blood derivatives

#### All Cell Pellet (ACP) and plasma EDTA

In order to optimize the blood collection, ACP and plasma EDTA are obtained from the same collection tube. The venous sampling is carried out using K3EDTA tubes. Blood is processed within 2 hours from collection. Tubes are centrifuged without brake at 3000rpm at RT (18–22 °C) for 15 minutes to separate the plasma from the cells. Using a micropipette, plasma is divided into at least 3x300 microliter aliquots in cryotubes and then transferred to -80 °C for storage as soon as possible. After removing the residual plasma, the tube is inverted two or three times to homogenize the sample. ACP is divided in 3x300-microliter aliquots in cryotubes and transferred to a -80 °C for storage as soon as possible.

#### Serum

The venous sampling is carried out using tubes with coagulation activator and gel separator. Blood is processed within 2 hours from collection and allowed to clot for a minimum of 15-20 minutes at RT (18-22°C) or until the clot is completely formed. Tubes are centrifuged at 3000rpm at RT (18–22 °C) for 15 minutes to separate serum from the cells. Using a micropipette, serum is collected without touching the separator gel with the pipette tip and divided into at least 3x300-microliter aliquots in cryotubes. Aliquots are transferred to a -80 °C freezer for storage as soon as possible.

#### Whole blood for total RNA extraction

The venous sampling is carried out using Tempus Blood RNA Tube (Applied Biosystems). Immediately after filling the Tempus tube, the blood is stabilized by vigorously shaking or vortexing the tube for 10-12 seconds. Samples are maintained at +4 °C for a maximum of 24 hours and then stored at -80 °C.

### Saliva Samples

Saliva is collected using Salivette Cortisol tube (Sarstedt) and the collection is carried out by the subject participant to the project according to the manufacturer's instructions.

Harvesting must be done in the morning and it is recommended:

- for at least 2 hours before harvesting:
  - not to eat
  - not to drink
  - not to smoke
  - not to take chewing gum

- to brush teeth at least 2 hours before the start of the harvest
- to avoid the use of cosmetic products for lips.

Samples are maintained at +4 °C and centrifuged within 1 hour from collection without brake at 3000rpm at RT for 15 minutes. Using a micropipette, the sample is divided into at least 3x300 microliter aliquots in cryotubes and stored at -80 °C within 2 hours from collection.

### **Stool Samples**

Stool sample is collected using DANASTOOL Sample Collection MICROBIOME Kit (DANAGEN) and the collection was carried out by the subject participant to the project according to the manufacturer's instructions.