RESEARCH PROTOCOL

BIOBANK DEVELOPMENT WITHIN THE NUTRINET-SANTE STUDY

Under the direction of:

Pilar Galan
DR1 INRA

Serge Hercberg
PU-PH University of Paris 13/Avicenne Hospital

INSERM U557/INRA 1125/CNAM/University of Paris 13

1. Biobank development within the NutriNet-Santé study

As initially designed, the NutriNet-Santé study is well positioned to serve as a databank for a sizeable amount of phenotypic data from a large population followed over time. In particular, data collection was planned to be Internet-based with vital status validation via medical records and use of national vital statistics registries (SNIIRAM, ALD, PMSI, CNAV). Initially, the study protocol did not include either the collection/storage of biological specimens or methods for direct validation of clinical events.

However, the addition of biological data collection (ie, blood and urine samples) to the protocol will greatly augment the capacity to analyze and test numerous mechanistic hypotheses (eg, genetic, proteomic, metabolomic, and biochemical), given the ample longitudinal data available.

2. Biobank objectives

The purpose of the NutriNet-Santé Biobank is to collect biological data from a subsample of participants, which will later allow a wide array of analyses. Such analyses will be aimed at shedding light on numerous mechanistic hypotheses within the nutrition-health relationship.

The development of the protocol regarding the collection, treatment and storage of biological samples takes into consideration the fact that there is no “ideal” way of addressing all potential scientific questions in all areas of research. In addition, it is not possible at present to determine the most useful biomarkers for disease detection. Finally, the protocol reflects technical, analytic, and financial limitations. For example, instead of 24h urine samples, spot urine samples are collected. Whereas no collection of saliva or hair samples is planned, this could be part of future ancillary protocols. Due to limitations, certain sample treatment methods (ie, stabilization at the Local Sample Collection Centers as, for example, treatment of vitamin C with metaphosphoric acid), or certain storage methods (eg, with liquid nitrogen) are also not planned.

For the most part, the Biobank protocol is based on the UK Biobank program (an ongoing national Biobank which includes 500 000 participants and 35 local centres). The selected criteria were defined following extensive consultations within the scientific community and a peer review. Then, a detailed instruction manual was put together in order to ensure that the selected methodology corresponds to the objectives of the Biobank. The Standard Operating Procedures manual of the UK center outlines in detail the methods of sample collection, on-the-spot treatment of the samples, their transportation under strict temperature requirements, the treatment at the central (coordinating) laboratory, the number of aliquots, and sample storage. This manual has been adapted to the needs of the NutriNet-Santé Biobank.

3. Population subsample

The purpose of the NutriNet-Santé Biobank is to collect, treat, and store biological data from a subsample of at least 20 000 NutriNet-Santé participants throughout the country. Standardized procedures as regards collection, treatment, transportation and storage of the samples have been implemented.

4. Logistics

4.1. Practical aspects of the organization

4.1.1. Centralized participant scheduling system

Study volunteers are invited to visit a participating Local Sample Collection Center (LSCC) equipped for blood sample draws (approximately 90 hospital-based LSCC has been opened, with a total of 4 LSCC being open at any one time).

An online, centralized participant invitation/scheduling system coordinated by U557 INSERM (University of Paris 13) is in place. It relies on a dedicated website accessible only by «Nutrinautes». Via that website, the participants receive all pertinent information regarding the objectives of the Biobank, conditions for the blood draws, sample volume, LSCC access, copies of the consent forms, etc.

An e-mail invitation with a built-in link is sent to the «Nutrinautes», allowing them to modify the date/time of the appointment. An automatic confirmation e-mail is sent once the appointment has been confirmed; an additional reminder e-mail is sent 48h before the appointment and includes information on permitted duration of fasting, permitted foods, etc. A final reminder e-mail is sent the day before the appointment.

In order to ensure acceptable participation rates, participants have the opportunity to take advantage of cholesterol (HDL and LDL), triglyceride, and diabetes screening tests (sending of the results for all participants with a special notice in case of abnormal results).

4.1.2 LSCC-based procedures and centralized specimen collection

The CLCP are in charge of receiving the participants and the field staff in charge of collecting the biological samples (blood and urine), collecting additional clinical data, on-the-spot treatment of the specimens, and transportation to the central laboratory for aliquot production and storage.

4.1.2.1. Coding of test tubes and urine collection vessels

The complete set of samples collected from each participant is placed in Vacutainer® tubes labeled with a barcode. The barcodes contain a sufficient number of digits to allow the following:

- De-identified and identical labeling of all samples (Vacutainer® tubes) for each volunteer;
- Identification of the sample collection containers (plastic tubes, EDTA K2 tubes, lithium-heparin tubes, urine collection vessels, etc.)

4.1.2.2. Types of test tubes

Blood samples are collected using the Vacutainer® system. A total of 43 ml of blood (ie, 5 vacutainers) is collected from each volunteer (Table 1). A spot urine sample is also collected in a vacutainer. A variety of tubes containing different kinds of anti-coagulants or separators are used. The anti-coagulants used (EDTA K2 and lithium-heparin) as well as the obtained sera will make feasible a variety of analyses. Blood from each volunteer are collected into: two 9 ml tubes containing EDTA K2, one 9 ml tube containing lithium-heparin, and two 8 ml plastic clot activator serum separation tubes. The order of sample collection has been set so that in case of difficulties in obtaining the full volume, the available draws could nonetheless permit a wide range of analyses (Table 1).

The various vacutainers are used to obtain plasma, serum, buffy coat (for DNA extraction) and red blood cells. The contents in each tube are in turn fractioned into a sufficient number of aliquots in order to permit various analyses to be carried out in the future. In addition, the lithium-heparin tubes and the plastic test tubes contain an inert gel which facilitates separation of the cellular components, thus preventing potential changes in plasma or serum during the interval between centrifugation at the LSCC and aliquot production at the central laboratory.

Type of sample (number of test tubes)	**Collection order**	**Volume collected/tube (ml)**	**Temperature until centrifugation and aliquot production (°C)**
EDTA K2 (1)	1	9	4
Lithium-heparin tube with plasma separation gel plug (1)	2	9	4
Plastic tubes with clot activator and serum separation gel plug (2)	3	8	4
EDTA K2 (1)	4	9	4
Total sample volume		43	
Urine (1)	-	9	4

Table 1 - Order of sample collection, volume and storage temperature until centrifugation and production of aliquots.

4.1.2.3. Test tube pre-analysis treatment in LSCC

All LSCC are supplied with refrigerated centrifuges to ensure the separation of plasma or serum within a set and standardized interval, which will prevent any degradation of the samples (eg, contamination from cell lysis if set interval is not observed).

4.1.2.3.1. Lithium-heparin tubes

The centrifugal process of these tubes is performed:

- Immediately after the draw
- Temperature: + 4°C
- Speed: 2 500 g during 10 minutes

The tubes are kept at + 4°C until shipment the same afternoon.

4.1.2.3.2. Plastic tubes

Following the draw, the plastic tubes are:

- Kept for 30 minutes at ambiant temperature in order to facilitate the coagulum retraction before the centrifuge process
- Temperature: + 4°C
- Speed: 2 500 g during 10 minutes

The tubes are kept at + 4°C until shipment the same afternoon.

4.1.2.3.3. EDTA K2 tubes

The two EDTA K2 tubes are kept at + 4°C without centrifugation. The actual treatment of these samples (ie, centrifugation, separation of plasma, buffy-coat, and red blood cells) is performed at the central laboratory immediately upon receipt of the tubes.

4.1.2.3.4. Urine collection vessels

Urine sample collection is performed on-site. These vessels allow the close-circuit urine transfer from the vessel to the Vacutainer® tube. This decantation is performed at the LSCC. The Vacutainer® tubes containing the spot urine sample are kept at + 4°C until shipment the same afternoon.

4.1.2.4. Operation surveillance at the LSCC

For each participant, the following information is entered in the Biobank database: date, time of sample collection, adherence to recommended fasting period, potential problems during blood draw, etc.

4.1.3. Transportation of specimens to central laboratory

Towards the end of every working day (Monday through Friday), each LSCC dispatchs the collected specimens in special parcels, to be delivered at the central laboratory the following day (Tuesday through Saturday). Transportation takes place overnight, to ensure the arrival of test tubes at the central laboratory by the early morning (before 9 am). The daily collection and shipment of the test tubes are carried out by a qualified courier, using special containers (meeting current standards) which ensure a temperature of + 4°C during transportation to the central laboratory.

4.1.4. Processing at the central laboratory

***Note**: Given the number of participants in the Biobank (60 per day) and the number of aliquots (30 per person, or 1 800 aliquots per day), manual aliquot production would be extremely challenging. The decision not to freeze the specimens at the LSCC presents organizational advantages (traceability, automatic aliquot production, etc..). Nonetheless, the interval between sample draws at the LSCC, the aliquot production at the central laboratory, and the storage at – 80°C could potentially lead to some negative consequences as regards certain labile molecules.*

The central laboratory is based at the Medical School of the University of Paris 13, adjacent to the NutriNet-Santé Biobank. Upon receipt, each barcode on the Vacutainer® tubes is scanned and matched with the data file from the LSCC in order to ensure that all test tubes collected the day before have arrived (a specimen check is also performed). An electronic connection between each LSCC and the central laboratory guarantees that all relevant information is available at the central laboratory even before the arrival and processing of test tubes. All transportation data are recorded at delivery.

A total of 30 aliquots are produced for each participant and stored at - 80°C. For security purposes, aliquots are stored in two separate freezers. The aliquot production is carried out by an automated system at + 4°C in order to ensure rapid and high-quality processing, to reduce the possibility of error, to ensure accuracy and traceability of all samples. The reason for which samples are rapidly fractioned into a large number of aliquots is to protect them from freeze-thaw degradation and to store them under optimal conditions for future analyses.

In total, 360 Vacutainer® tubes are treated daily in the central laboratory (ie, 60 participants/day for the 4 LSCC, with 6 Vacutainer® tubes (blood and urine) per person). Upon receipt in the laboratory, the EDTA tubes are centrifuged at 2 500 g during 10 minutes at + 4°C. The tubes are treated with the aim that all samples are cryopreserved not more than 24h after collection (as shown in Table 2).

The aliquots produced from the Vacutainer® tubes are stored in cryogenic tubes especially engraved with 2-dimensional (2D) barcodes. Each day, a total of 1 800 aliquots are produced (ie, total volume of 850 µl). The process of aliquot production is fully automated to ensure accurate identification of the 2D-labeled tubes and to guarantee traceability from the sampling to the archiving (in the deep freezers) in the Biobank.

Vacutainer tube	Fractions	Number of aliquots frozen at -80°C
EDTA K2 x 2	Plasma	8
	White blood cell layer (buffy coat)	2
	Red blood cells	2
Lithium-heparin with plasma separation gel plug	Plasma	4
Plastic tubes with clot activator and serum separation gel plug x 2	Serum	6
Urine	Urine	8
Total number of aliquots		30

Table 2 - Fractions and aliquots from the Vacutainer® tubes

4.1.5 Specimen storage

The various aspects of the Biobank (ie, quantity of tubes to be frozen, rigorous surveillance) necessitate the use of special software (LIMS) operated from the LSCC, the central laboratory and the storage unit at the Biobank.

Storage trays contain 96 cryogenic tubes with 2D barcodes, which are scanned in the automated system in order to track their exact location in the - 80°C freezers (compartment, rack and position). For security reasons, the aliquots from each participant are stored in two separate freezers at the Biobank at the University of Paris 13 (Bobigny). Each freezer contains aliquots (properly organized in racks) from approximately 2 700 persons. Two additional, empty freezers are available for use in case of technical problems (ie, additional security in case a freezer goes out of order). All freezers are connected to temperature detectors which, in turn, are connected to a telephone and electronic alarm system. This ensures constant temperature surveillance and immediate response in case of a power outage. The Biobank is equipped with a fire protection system, a trespassing alarm, and an engine-generator for use in case of a massive power outage.

This security system allows contacting by telephone the responsible personnel in case the temperature of a freezer exceeds the set degrees. The telephone alarm system is connected 24/7 to the cell phones of the responsible Biobank personnel and to a company for urgent freezer repair.

The Biobank is physically located on the underground level of SMBH at UREN, in an area made available for that purpose by the University of Paris 13. The premises consist of a big

hall of 250 m² to house the freezers (a total of 40 freezers can be maintained) and the central laboratory. The Biobank is air-conditioned.

5. Organization and management of the Biobank

The principal investigator of the NutriNet-Santé study is Pr Serge Hercberg, Director of UMR INSERM U557/INRA 1125/CNAM/University of Paris 13.

The Biobank is under the responsibility of Dr. Pilar Galan (DR1 INRA, UMR INSERM U557/INRA 1125/CNAM/University of Paris 13).

Field staff
The field staff consist of 4 laboratory technicians (1 per LSCC), each managing 15 participants per day.

All individual-level Biobank data are centralized at UMR INSERM U557/INRA 1125/CNAM/University of Paris 13 and are monitored by the NutriNet-Santé study coordinators. These data are linked with the sociodemographic/dietary data (while observing the principles of de-identification, data protection and security, in accordance with current legislation policies).

6. Regulatory aspects

The NutriNet-Santé study (including participant recruitment, data collection via the Internet, etc.) has received approval from the INSERM's «Comité de Qualification Institutionnel» (dated 10/07/2008), from CCTIRS (dated 11/07/2008, n°08.301) and from CNIL (dated 24/02/2009, n°908450).

The Biobank conforms to all legislation regulations in France, such as: reporting the collection to ARH at the Ministry of Research (article L 1243-3; order n° 2007-1220), approval by AFSSAPS, CCP, CCTIRS and CNIL. The INSERM Public Health Institute has given its accord for INSERM's support for the study (file n° C09-42, 22/12/2009). The project has also been approved by the CCTIRS (dated 15/07/2010, n°10-367).

All procedures regarding the function and quality control of the NutriNet-Santé Biobank rely on the following:
- Recommendations published in NF S96-900 from July 2008 on the "Quality of Biological Resource Centers (CRB) - Management System of a CRB and Quality of Biological Resources from Human and/or Microorganism Origin."
- OCDE guidelines regarding best practices among biological resource centers (OECD, April 2007).