EPHOR Exposome tools for a healthy working life

Exposome project for health and occupational research

Protocols for collection, pre-processing and storage of biological samples (WP3, Task 3.1)

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WP (number and title)	WP3- Internal exposure and effect assessment using biomonitoring, omics and minimally invasive biomarker development
Deliverable Number	D3.1
Deliverable Title	Protocol for the collection, pre-processing and storage of biological samples in WP6, WP7
Due date	Month 6
Actual date	
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Lead beneficiary	6 - KUL
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Co-authors	WP3, WP6 and WP7 partners

Collection, pre-processing and storage of blood

Introduction

Blood samples will be used for metals, cotinine, white blood cell count, cytokines, hydroxydeoxyguanosine, mitochondrial DNA copy number, telomere length, GWAS, epigenomics, targeted RNA expression, pyrosequencing, cell-free DNA methylation, proteomics and fluorescent oxidation products (FlOPs). Exposure to metals can result in oxidative damage. Therefore, Pb and As and possible other metals (e.g. Cd, Hg, Pt, Fe, Mn) will be measured in blood. Measuring cotinine gives an insight into the smoking behavior of the participants and will be done for samples from WP6 (asthma/COPD short-term study) and WP7 (shift workers). Furthermore, epigenome-wide association studies (EWAS) help elucidate associations between altered DNA methylation and health outcomes as well as environmental impacts on DNA methylation which is integral to the EPHOR study (WP6 and WP7). DNA methylation will additionally be studied using bisulfite Pyrosequencing. It is a technique based upon the "sequencing by synthesis" approach. It provides quantitative and highly reproducible methylation data at single-base resolution, and it requires relatively low quantities of DNA. This technique will be applied for samples from WP6 and 7. In addition, efficiently identifying circulatory proteins may provide a more mechanistic overview associated disease phenotypes, and at the same time help in development of predictive biomarkers (WP6). In this context another cell free marker, the cell-free DNA (cfDNA) in blood plasma is emerging as a powerful tool for disease diagnostics and biomarker development. And therefore, epigenetic marks found on cfDNA, such as DNA methylation, can be used as biomarkers for exposure and disease and will be validated in the present study (WP6). Moreover, 8-hydroxydeoxyguanosine (8-OHdG) will be used for measurement of oxidative damage. Blood samples from participants in WP6 and 7 cohorts will serve as matrix for 8-OHdG quantification. In addition, also FlOPs will be evaluated. FlOPs are a global marker of oxidation processes, including protein and DNA oxidation and lipid peroxidation are of growing interest in epidemiology. This biomarker of damage has been found to be associated with chronic diseases including asthma. Furthermore, relative mitochondrial DNA copy number (mtDNAcn) and relative telomere length (rTL) will both serve as biomarkers for senescence/DNA damage. In addition, mtDNAcn will be used for the assessment of oxidative stress, whereas rTL will be used for the assessment of biological ageing in relation to occupational exposure. mtDNAcn and rTL will be assessed in cases and controls from WP6 and shift workers from WP7. Moreover, digital droplet (dd)PCR provides high-precision, absolute quantification of nucleic acid target sequences in a wide-range of applications including analysis of gene expression, microRNA analysis and genomic alterations such as copy number variations (CNV). ddPCR will be used to assess biomarkers for occupational exposure to shift work and other exposures i.e. stress, etc. (WP6 and 7). Finally, investigations of the normal sleep–wake cycle showed that the production of pro-inflammatory cytokines exhibit peaks during early nocturnal sleep whereas the anti-inflammatory cytokine activity peaks during daytime wakefulness. Consequently, mistimed sleep and the associated alterations of circadian rhythms (like in a night shift work) are suggested to lead to disturbed immune responses which might contribute to the increased risk for infection, autoimmune diseases, cardiovascular and metabolic disorders, and cancer. A panel of 30 cytokines, chemokines and growth factors will be measured in plasma samples to assess changes in their profile in night shift workers compared to day shift workers. Concentrations will be measured at multiple time-points for several days based on feasibility in all contributing cohorts. Certain other parameters (e.g. IgE, CC16, YKL40) that have an added benefit for the study could be measured in the samples that will be collected during the project.

Note: The exact time of sampling will be discussed with WP6 and 7 leaders.

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Materials needed

Collection:	-10 mL Vacutainer® EDTA tubes -2.5 mL PAXgene® blood RNA tube -6 mL Vacutainer® blood collection tube for trace element testing -rack for tubes -BD Vacutainer® Safety-Lok blood collection set -Powder-free disposable gloves -70% alcohol swabs for skin disinfection -Garrottes/tourniquets -Adhesive bandages or tapes -Container for disposal of used needles after venipuncture -Labels: country ID – participant ID – sample ID (including date and time of collection) -Barcode scanner -Datasheet for information on time of sample collection and time since last meal
Pre-processing:	-Bench top Centrifuge (Eppendorf/Sigma) -Pipettes and tips -Nalgene™ General Long-Term Storage Cryogenic Tubes (Catalogue number: 5000-0020; Volum e- 2 mL; ThermoFisher) -Labels: country ID – participant ID – sample ID (including date and time of collection)
Storage:	-Storage box -Freezer -80 °C

Collection of blood

Note: Clean space preferable; some experiments need sterile condition.

Participants are asked to refrain from drinking, eating and smoking before collection.

Type of tube	**Number of tubes**	**Volume of tubes (mL)**
Vacutainer® EDTA	3	10
PAXgene® blood RNA tube	1	2.5
Vacutainer® blood collection tube for trace element testing	1	6
Total volume		38.5

Note: Additional clinical parameters might be studied, thus an additional heparin tube might be collected. This will be added to the protocol after discussion with the clinical lab.

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Instruction for collection

- Label tubes
- Select tube for sample collection (first BD Vacutainer® EDTA, then BD Vacutainer® blood collection tube for trace element testing, end with PAXgene tube). Place sample tubes on a rack in order of collection.
- Blood samples are collected according to WHO's best practices in phlebotomy.
- Assemble a blood collection set with 12-inch tubing into a BD Vacutainer® One Use Holder. Be sure that blood collection set is firmly attached to holder and does not unthread during use.
- Hands are washed and a mask is worn. Patients are identified and prepared.
- Select site for venipuncture; Apply tourniquet.
- Prepare a venipuncture site with appropriate antiseptic. Do not palpate the venipuncture site after cleansing.
- Perform venipuncture with limb downward and tube stopper up (for prevention of backflow).
- Push tube onto non-patient-end (NP-end) of needle in one swift action. Hold tube on NP-end during drawing.
- Remove tourniquet as soon as blood appears in the last tube.
- Do not allow the contents of the tube to contact the stopper or end of the needle during the procedure.
- Allow vacuum to be exhausted prior to removing the tube from the NP (non-patient) end of the needle.
- Give the participant an adhesive bandage or tape to apply to the puncture site.
- Discard the used equipment into a puncture-resistant container, discard sharps and broken glass into the sharps container and discard items containing blood or body fluids into the infectious waste.
- Remove gloves and wash hands.
- Mix specimen tubes with additives, by slowly inverting the tube 8 to 10 times immediately after blood collection.
- Fill-in datasheet with information on time of collection and covariates.

Pre-processing and storage of blood at site of collection

Note: sterile condition required for most of the following steps.

Time allowed from collection and processing: 2-6 h.

Note: It is very important to minimize the time between collection and separation of plasma; We want to study cell free DNA/ protein and prolonged storage of blood after collection without processing will result in haemolysis and cell death; Thereby, resulting in release of cellular DNA and proteins into the matrix and altering the profile.

- 1 PAXgene RNA tube of 2.5 mL is kept for 1 h at ambient temperature and then stored at -80 °C for ddPCR analysis.
- 1 Vacutainer® blood collection tube for trace element testing of 6 mL is X.
- Blood from EDTA tubes is aliquoted in 6 cryo-vials of 2 mL (1 mL per vial). 2 vials are sent for differential count of cells at the site of collection, 4 vials are immediately stored at -80 °C.
- The remaining blood samples from EDTA tubes are centrifuged (2000 x g, 10 minutes, at 20 °C). (**Note**: Shorter time between collection and centrifugation should be ideal)

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- After centrifugation, plasma is aliquoted in 8 cryo-vials of 2 mL (1 mL per vial). All vials are carefully labeled.
- Store all cryo-vials at -80 °C.
- Leave the cellular fraction (pellet) in the EDTA tube and store at -80 °C.

Note: Please note that the final method for cotinine will be adapted based on method development, optimization and validation. Before the start of the experiments, the developed and validated protocol will be made available. The protocol for collection, pre-processing and storage of blood for cotinine analysis described here is therefore only a limited outline.

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