HBM4EU

science and policy
for a healthy future

# HBM4EU project

## Biomarkers in human biological monitoring

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1. Introduction – Human biomonitoring
2. Human biomonitoring vs. Environmental monitoring
3. Biomarkers of exposure, effect (response) and susceptibility
4. From biological samples to biomarker
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# *Human biomonitoring (HBM)*

## Definition:

- HBM is a scientifically developed approach for assesing human exposures to natural and synthetic compounds from environment, occupation, and lifestyle
- Biological monitoring is defined as the repeated, controlled measurement of chemical or biochemical markers in fluids, tissues or other accessible samples from subjects exposed (currently, in the past or to be exposed) to chemical, physical or biological risk factors in the workplace and/or the general environment

- **Aim: identification of markers of long-term risk**
  - Humans – carcinogenesis, occupational diseases
  - Ecotoxicology – early markers of toxic effects

Ladeira and Viegas, Biomonitoring 2016;3:15-24.

# *Human biomonitoring (HBM)*

## Applicability:

- to develop and validate biomarkers that reflect specific exposures
- to provide exposure and risk information
- to predict the risk of disease in individuals and in population groups
- to inform public health decisions and/or initiate policy measures
- to focus on the protection of susceptible populations, such as children and pregnant mothers
- to provide data for screening
- to provide data for prioritization of chemicals for further research or regulation

## Examples:

- ban of lead from gasoline
- avoidance of mercury-containing amalgam teeth fillings in children
- restriction of phthalate use in plastics

Ladeira and Viegas, Biomonitoring 2016;3:15-24.

# *Human biomonitoring vs. Environmental monitoring*

## Biomonitoring advantages

- Biological samples reveal integrated effects of repeated exposure
- Biomonitoring data **directly reflect total body burden or biological effects** resulting from all routes of exposure: inhalation, absorption through the skin, and ingestion
- Biomonitoring data also reflect modifying influences in physiology, bioavailability, bioaccumulation and persistency
- Persistency can magnify concentrations of some environmental chemicals (e.g. persistent organic pollutants, and metals such as lead and cadmium) enough to raise them above detection thresholds

## Environmental monitoring

- periodic measurement of the level or concentration of a chemical, physical or biological risk factor in the workplace / environment, which is traditionally used as an **indirect measure of human exposure**.
- is crucial for development of targeted policy actions

# *Biomarkers*

Biomarkers are indicators signaling events in biological systems or samples

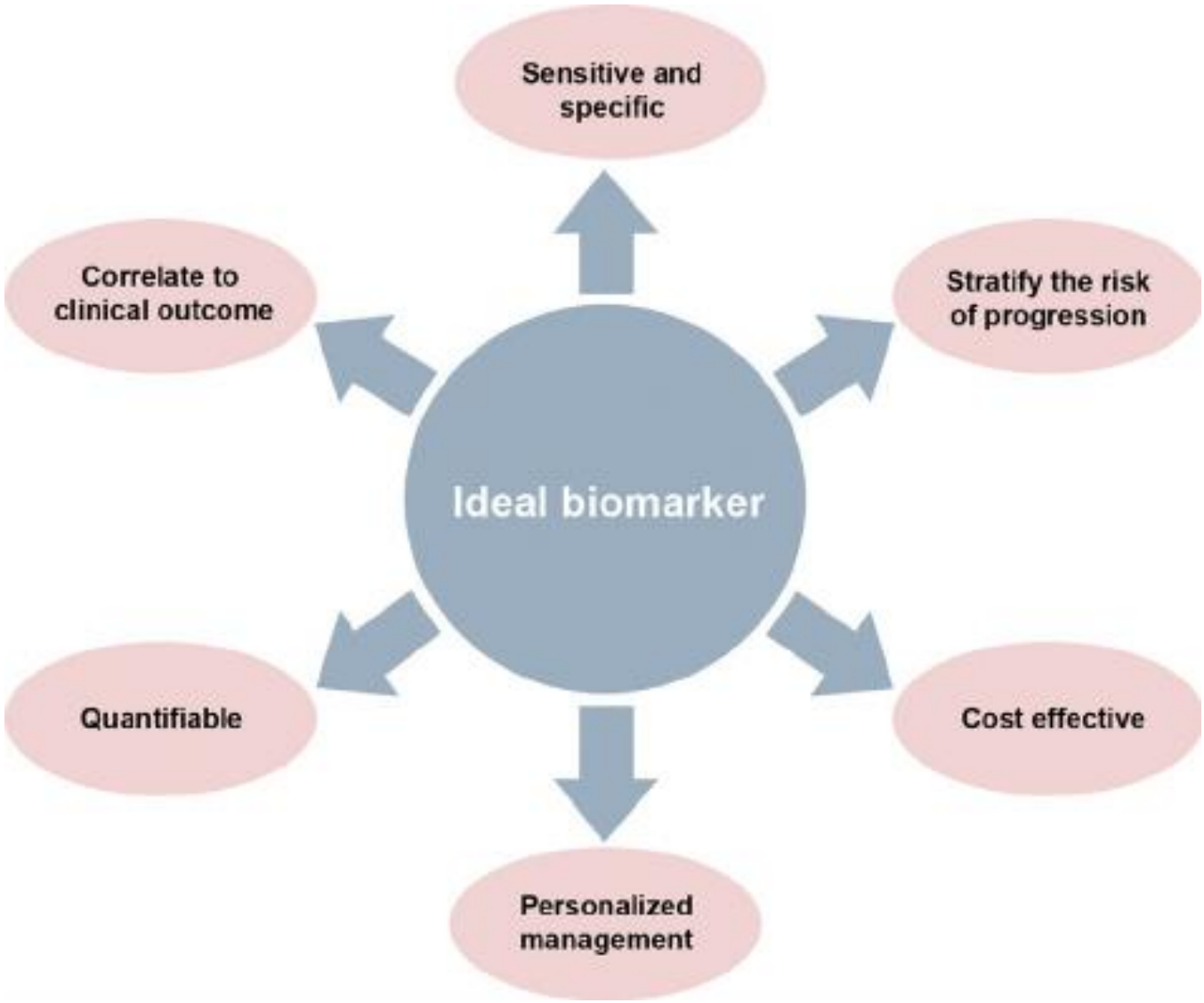
## Definition (U.S. National Academy of Sciences Committee on Biological Markers):

- Markers in biological systems with sufficiently long half-life which allows identification of location **WHERE** in the biological system changes occur and **QUANTIFICATION** of the changes
- A biomarker can be **any substance, structure or process that can be monitored in tissues or fluids and that predicts or influences health**, or assesses the incidence or biological behavior of a disease
- alteration in cellular or biochemical components, processes, structure or functions that is measurable in a biological system or sample, but is not a measure of the disease, disorder or condition itself.

## Identification of markers of long-term risk:

- Humans - carcinogenesis
- Ecotoxicology – early markers of toxic effects

# *Ideal biomarker*



# *Categories of biomarkers*

## Biomarkers of exposure :

- "exogenous substances (xenobiotics) or their metabolites or the products of interaction between a xenobiotic and target molecule or cell that is measured in a compartment within an organism (NRC, 1989)

## Biomarkers of effect:

- a measurable alteration in an organism (biochemical, structural, functional, behavioral or any other)that, according to its magnitude, can be associated with established or potential health impairment or disease

## Biomarkers of susceptibility :

- an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a chemical or xenobiotic substance

Allocation of a biomarker to one type or the other may depend on its toxicological significance and the specific context in which the test is being used

Ladeira and Viegas, Biomonitoring 2016;3:15-24
Pohl et al .Toxicology and industrial health 2000; 16: 3-5.

# *Biomarkers of exposure*

- identify and measure chemical residues in tissue or body fluids, metabolites of xenobiotic compounds, or physiological outcomes that occur as a result of exposure

## The role of biomarkers of exposure in occupational health

- to assess exposure by all routes and to complement information obtained by workplace environmental monitoring.
- may be used as a better substitute for environmental monitoring for indicating exposures to environmental pollutants which are important to public health
- Exposure biomarkers can reflect bioavailability and be influenced by numerous parameters such as route of exposure, physiological characteristics of the receptor, and chemical characteristics of the xenobiotic

# *Biomarkers of exposure*

## Markers of internal dose

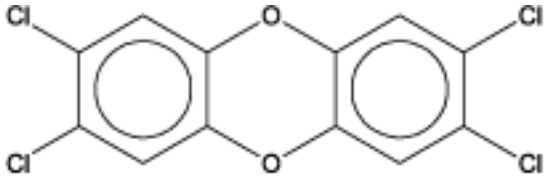
- give an indication of the occurrence and extent of exposure of the organism, and thus the likely concentration of a parent compound or metabolite at the target site.
- Example*:* **blood concentration** of a chemical agent measured **following exposure**

## Markers of effective dose

- indication of the true extent of **exposure of the target molecule, structure or cell**.
- Example*:* target enzyme inhibition or induction, **lipid peroxidation**, **DNA damage**

- Both are preferable to measuring external levels of the compound in question (for example in the workplace), as they take into account biological variations in absorption, metabolism and distribution of the compound between individuals

# Biomarkers of exposure to TCDD



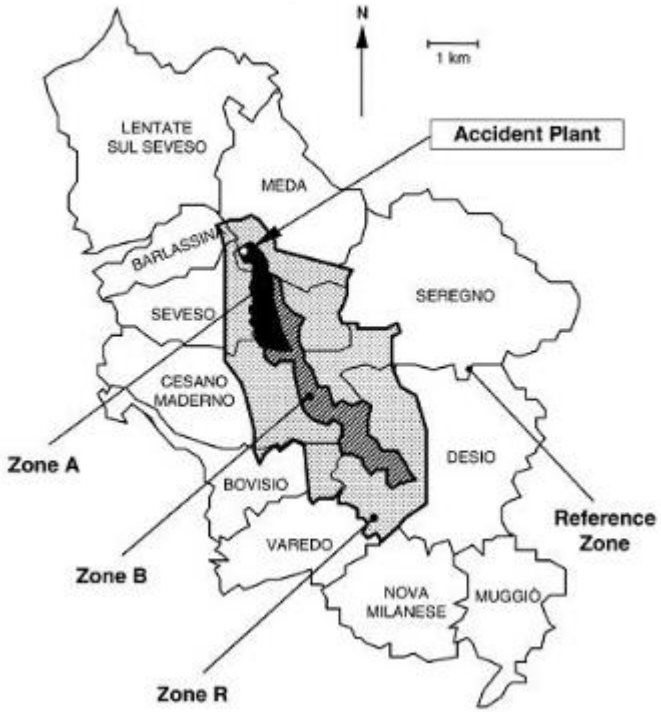

2,3,7,8-tetrachlorodibenzo-*p*-dioxin

## Seveso Accident, 1979

- The **Seveso disaster** was an industrial acciden that occurred around 12:37 pm July 10, 1976, in a small chemical manufacturing plant approximately 15 km (9.3 mi) north of Milan in the Lombardy region in Italy.
- It resulted in the highest known exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in residential populations which gave rise to numerous scientific studies and standardized industrial safety regulations.

2

# Biomarkers for quantification of exposure to TCDD




| Zone | Mean soil level (μg/m²)‡ | Median lipid-adjusted serum level in pg/g or parts per trillion | |
|---|---|---|---|
| | Minimum–maximum | Samples collected in 1976–1977§ (no.) | Samples collected in 1992–1996¶ (no.) |
| A | 15.5–580.4 | 447.0 (296) | 73.3 (7) |
| B | 1.7–4.3 | 94.0 (80) | 12.4 (51) |
| R | 0.9–1.4 | 48.0 (48) | NA* |
| Reference | NA | NA | 5.5 (52) |

* TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; NA, not available.
† Refer to the Materials and Methods section of the text for a definition of the study zones.
‡ Refer to Bertazzi and di Domenico (26) for more information.
§ Refer to Needham et al. (43) for more information.
¶ Refer to Landi et al. (41) for more information.

TABLE 2 . TCDD* soil levels and serum concentrations measured in selected samples of residents in the study areay after the 1976 Seveso, Italy, accident

Consonni D et al. American journal of epidemiology 2008;167(7):847-58

# *Effects of TCDD poisoning*

## Acute effects:

- Chloracne
- Nausea
- Vomiting
- Epigastric pain
- Appetite loss
- Weight loss
- Abnormal liver tests
- Increased abortion rate

## Long term effects:

- Abnormal sex ratio (more girls) in relation to paternal exposure.
- TCDD- concentration in breastmilk after 25 years still twice as high
- Increase in all cancers (rectum, lympho-hemopoietic, myeloid, thyroid gland and pleura)

# *Biomarkers of effect (response)*

## Definition (International Programme on Chemical Safety)

- "a measurable biochemical, physiological, behavioral or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease"

### Biomarkers of effect in occupational health

- They result from interaction of the organism with different environmental factors (including chemical, physical, and biological agents).
- They can be measured at the level of the whole organism, at the level of organ function, at the level of tissue and individual cells, and at the subcellular level
- They can be used for monitoring of disease progression and prognosis, and as adjuncts to other biomarkers in providing refinements in epidemiology and risk assessments.
- They may provide information on proposed exposure-disease pathways in vivo in human populations.
- They may be useful for demonstrating the biologic influence of preceding susceptibility factors - for instance, genetic polymorphisms of xenobiotic-metabolizing enzymes
- If validated, they may be used as early predictors of clinical disease to improve occupational health risk assessment and contribute to implement new effective disease prevention policies in occupational and environmental settings

# *Biomarkers of genotoxicity*

- Used to assess **exposure to genotoxic agents**
- Indicators of **early events** associated with disease-related changes, in particular early carcinogenic effects
- May be used to measure specific occupational and environmental exposures, to predict the risk of disease, or to monitor the effectiveness of exposure control procedures to genotoxic chemicals
- **Cytogenetic biomarkers** are the most frequently used endpoint in human biomonitoring studies, and are used extensively to assess the impact of environmental, occupational and medical factors on genomic stability
- **Lymphocytes** may be used as a surrogate for the actual target tissues of genotoxic carcinogens ;
- **Genotoxicity biomonitoring endpoints:** micronuclei, chromosomal aberrations, 8-hydroxydeoxyguanosine (8-OHdG) and DNA repair measured by comet assay

Franko et al., J Biomed Biotechnol, 2009
Franko et al., J Arh Hig Rada Toksikol, 2008
Franko et al., J Biomed Biotechnol, 2011

# *Biomarkers of susceptibility*

## Definition (NRC,1989)

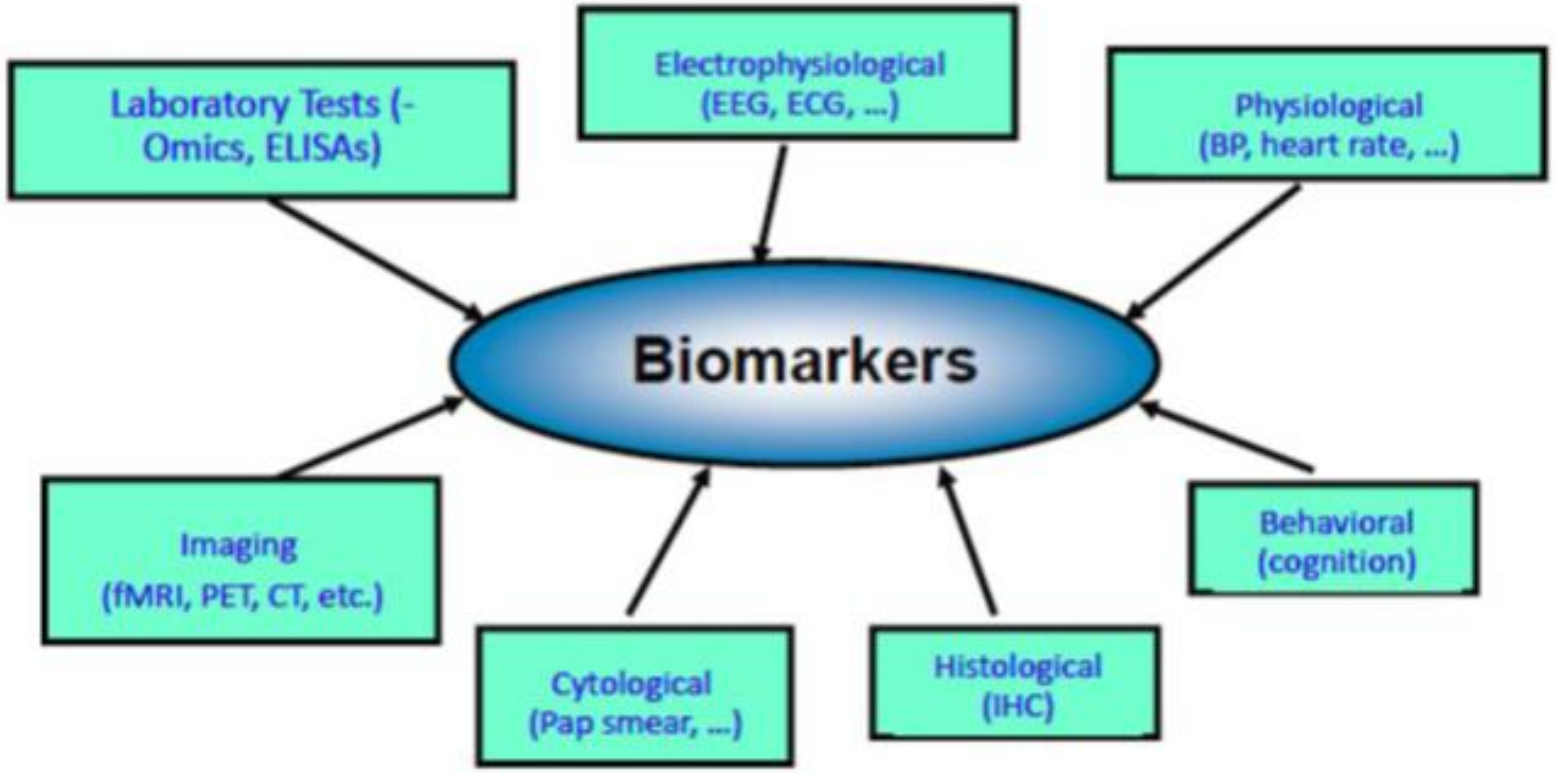
- indicator or a measure of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance or develop disease

### Biomarkers of susceptibility in occupational health

- any variation in the response of an individual to identical exposures may lead to difference in susceptibility, due either to the **genetic make-up of the individual** or to **variables and environmental and lifestyle influences**, such as diet, smoking, alcohol
- Variability in the uptake, absorption, distribution, metabolism (detoxification) and excretion of xenobiotics (ADME)
- Variability in target molecules for toxic chemicals
- Variability in defence and repair systems such as antioxidative defence, protein degradation or DNA repair mechanisms

# *Biomarkers of susceptibility*

- Biomarkar may be a protein, RNA, gene, laboratory characteristic, clinical characteristic, imaging ….



# *Interindividual variability of susceptibility*

- individuals can often differ markedly in their qualitative and quantitative responses to chemical exposure
- Such interindividual differences can be genetically mediated, or can be the result of some environmental stressor, lifestyle, disease process or other epigenetic factor.
- These mey lead to different levels of expression of genotypic variations in at risk phenotype
- these interindividual differences can also be usefully employed as biomarkers of individual susceptibility to xenobiotics
- Susceptibility markers are useful because they can partially explain interindividual variation inherent in the general population, and thus provide a biological rationale for investigation of inherent vulnerability prior to exposure to environmental hazards

Franko et al., J Biomed Biotechnol, 2009
Franko et al., J Arh Hig Rada Toksikol, 2008
Franko et al., J Biomed Biotechnol, 2011

# *Biological samples and biomarkers*

## Biologic samples

- blood
  - plasma/serum
  - exosomes
- saliva, bucall swabs
- tissue
  - frozen tissue
  - FFPE
- Bone marrow smears

## Biomarkers:

- DNA
  - SNP
  - Rare mutations
  - CNV
- RNA
  - mRNA, miRNA
- proteins

# *DNA as biomarker*

## blood

- **advantages**
- High quantity
- Best quality
- Simple extraction procedures

- **limitations**
- Venepuncture
- Patients has to be reffered to a laboratory

## FFPE

- **advantages**
- Normal and/or tumor tissue
- Archived FFPE or bone marrow smears
- Retrospective studies

- **limitations**
- fragmented DNA
- crosslinking
- Complicated extgraction procedures
- Limited amount of the material

## Bucall swab, saliva

- **advantages**
- Neninvasive, simple procedure to obtain a sample
- Patient can take a sample at home and mail it to the lab
- Simple extraction procedures

- **limitations**
- Smalled DNA quantities
- DNA can be fragmented

# *RNA as biomarker*

| blood | FFPE | Frozen tissue |
|---|---|---|
| • **advantages**<br>• Simple extraction<br>• Easy translation to clinical practice<br>• Can determine changes in expression levels<br>• Plasma miRNA more stable than RNA<br><br>• **limitations**<br>• Tissue specific expression levels<br>• Low quantities ofmiRNA in serumu/plasmarazgradnja RNA<br>• Extraction from whole blood / lymphocytes / frozen blood samples | •**advantages**<br>•Tissue specific expression levels<br>• Allows comparison of expression levels in normal and tumor tissue<br>•Archived samples allow for higher numbers of patients in the retrospective studies<br>•miRNA more stable than RNA<br><br>•**limitations**<br>• fragmented mRNA<br>• crosslinking<br>• More complicated extraction procedures<br>• Limited amount of sample | • **advantages**<br>• Tissue specific expression levels<br>• Allows comparison of expression levels in normal and tumor tissue<br>• miRNA more stable than RNA<br><br>• **limitations**<br>• Degrading enzymes – stabilization agents needed<br>• Fast processsing necessary |

# *Proteins as biomarker*

**serum/plasma**

- **advantages**
- Simple sample prepatation
- Simple measurment (npr. ELISA)
- Simple translation to clinical practice
- Allows monitoring of temporal changes in expression levels

- **limitations**
- Is not a good surrogate for expression levels in different tissues

**FFPE**

- **advantages**
- Allows monitoring of expression in a particular tissue
- Allows comparison of expression levels in normal and tumor tissue
- Archived samples allow for higher numbers of patients in the retrospective studies

- **limitations**
- Limited amount of materials
- Immunochemistry based assays may not work (epitope lost in denatured protein)

# *Biomarker identification technologies*

## Biomarker Identification technologies

High throughput technologies

1. Genomics
   1. Genome sequencing
   2. Genome variation
   3. Genome annotation
2. Transcriptomics
   1. Microarrays
   2. Gene expression data
3. Proteomics
   1. Y2H method
   2. Mass spectrometry
   3. Protein chips
4. Metabolomics
   1. NMR
   2. Mass spectrometry
5. Other technologies-
   1. Fluorescent indicators
   2. Lab-on-chip
   3. Nuclear magnetic resonance
   4. Mass spectrometry/liquid chromatography
   5. Nanobiotechnology
5. Imaging

# *Sampling*

## Time component important for the quality and comarison of samples and studies

- Depends on the  aim and the protocol of the study
- Needs to be determined before the beginning if the study

## DNA based SNPs

- Timing not important
- FFPE – normal  tissue and not tumor tissue
- (somatic mutations in tumor tissue!)

## RNA/miRNA/other biomarkers

- Timing important

# *Sample procesing and storage*

## How long ?

What will be the use of the stored samples?

## Where and at what temperature?

- blood/tissue/DNA/RNA/miRNA
- 4 °C/ -20 °C/ -80 °C

## Biobanking

Baker. (2012) Nature. 486, 141–146.

# *Sample analysis*

## DNA extraction and analysis

- germline, somatic, mitochondrial DNA
- Targeted, exome, genome analysis

## (mi)RNA extraction

- soluble, contained in exsosomes/cells/tissue
- qPCR, microarrays, sequence based analysis

## protein extraction

- soluble, contained in exsosomes/cells/tissue
- enzyme activity measurments, ELISA
- proteomics, mass spectrometry, protein chips

## Metabolite extraction

- Biochemical assays, HPLC, NMR, mass spectrometry

# *Limitations and social, ethical, and legal implications of susceptibility biomarkers*

- The study of susceptibility in human populations poses a number of ethical challenges.
- Ethical considerations should always be borne in mind before biomonitoring programs are planned and implemented, particularly when new or partially validated biomarkers are involved.
- The strategy for data handling, data analysis, interpretation, communication, and dissemination of the results to the workers, comparison groups, and others involved in the process should be considered in the beginning of any biomonitoring planning
- Since the primary purpose of biological monitoring is the protection of workers' health, situations must be avoided where the data gathered from exposure, effect or susceptibility biomarkers could result in an adverse impact on a worker's status of employment and/or quality of life
- In principle, biological monitoring should not result in discrimination or reduction of job opportunities for the workers involved.

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## Contributers

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### Speaker's information

Vita Dolžan, MD PhD, spec.med.lab.genet., works as a full professor of Medical biochemistry and molecular genetics at the University of Ljubljana, Faculty of Medicine (UL MF) and is the head of the Pharamacogenetics laboratory at the Institute of Biochemistry UL MF. Her main areas of research interest are pharmacogenetics, molecular genetics and molecular epidemiology. She is collaborating with members of HBM4EU WP2.

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