Deliverable D6.5

Project Title:	Developing an efficient e-infrastructure, standards and data-flow for metabolomics and its interface to biomedical and life science e-infrastructures in Europe and world-wide.	
Project Acronym:	COSMOS	
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Deliverable title:	Report on the recommendations of the use of Metabolomics for Biobank sample monitoring	
WP No.	6	
Lead Beneficiary:	10: CIRMMP	
WP Title	Coordination with BioMedBridges and biomedical ESFRI infrastructures	
Contractual delivery date:	01 10 2014	
Actual delivery date:		
WP leader:	Prof. Claudio Luchinat	CIRMMP
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Content

1. Executive summary

This deliverable describes and summarizes a number of different activities that contribute to defining and supporting the role of metabolomics as a tool to validate preanalytical procedures for sample collection and storage in biobanks as well as to quantitatively assess the stability of stored samples over the years. For example, metabolomics demonstrated the impact of medical procedures prior to human sample collection, e.g. as a consequence of anesthesia or antibiotic treatment. Guidelines based on the information described here have been formulated in the form of a document currently under evaluation by CEN for approval as a European standard. This initiative started within the European project SPIDIA.

To take up on the above opportunities for the development of metabolomics as a crucial technique to flank the development of standard operating procedures and quality control in biobanks, a Expert Center for Metabolomics (EXCEMET) has been formally established (http://www.excemet.org/). EXCEMET proposes itself as a reference infrastructure for biobanks and has been described in a recent concept paper by the now established BBMRI-ERIC as a model of possible BBMRI-ERIC Expert Center.

2. Project objectives

With this deliverable, the project has reached or the deliverable has contributed to the following objectives:

No.	Objective	Yes	No
1	Coordinate with the activities of BiomedBridges regarding the standardization of metabolomic data		x
2	Coordinate the activities of COSMOS, taking into account the requirements of Biobanks with respect to the association of NMR metabolic profiles to stored samples.	X	
3	Develop a strategy for the use of Metabolomics for Biobank sample monitoring and deposition of the metabolomics experiment data in COSMOS partner databases, particularly MetaboLights	X	
4	Offer advice and guidance and be receptive for any information relating to standardisation, policy and regulatory, EU Member	X	

	States initiatives or relevant international initiative.		

3. Detailed report on the deliverable

3.1 Background

The aim of WP6 is to foster the interaction between COSMOS and the biomedical infrastructures with a particular interest in metabolomics (BBMRI, Elixir, EU-Openscreen, EuroBioimaging and INSTRUCT) that are also participating in the BioMedBridges project. The idea is to obtain indications useful to focus and prioritize the various activities in COSMOS in order to effectively respond to the needs of the current large scale EU biomedical infrastructures.

The present document reports on the usage of metabolomics as a tool to enable sample monitoring in biobanks. We also describe the key role of metabolomics in validating standard operating procedures for the preanalytical workflow.

3.2 Description of Work

3.2.1 Metabolomics as a tool for biobanks

Research biobanks collect, handle, store and distribute biological samples and associated data for studies in the biomedical field. Most of this material is intended to be analyzed mainly by omics technologies. In particular, the ability to obtain quantitative information from a molecular profile is an exceptionally powerful means to explore basic biology, to diagnose disease, to facilitate drug development, to tailor therapeutics to specific pathologies or genetic profiles, and also to generate databases relevant to biological or therapeutic processes and pathways. The reliability of the resulting outcomes is strictly dependent on the preservation of the intactness of the original molecular profiles throughout the preanalytical workflow (Figure 1). On the other hand, the lack of guidelines in

collection, handling, transport, stabilization and storage of biosamples inevitably limits the reproducibility of subsequent analyses and thus their value. Biobanks are the institutions/infrastructures in charge of the preanalytical phase, partly because some of these steps occur within the biobanks themselves but largely because it is their responsibility to make available to sample providers clear operating procedures that guarantee the quality of the samples to be stored.

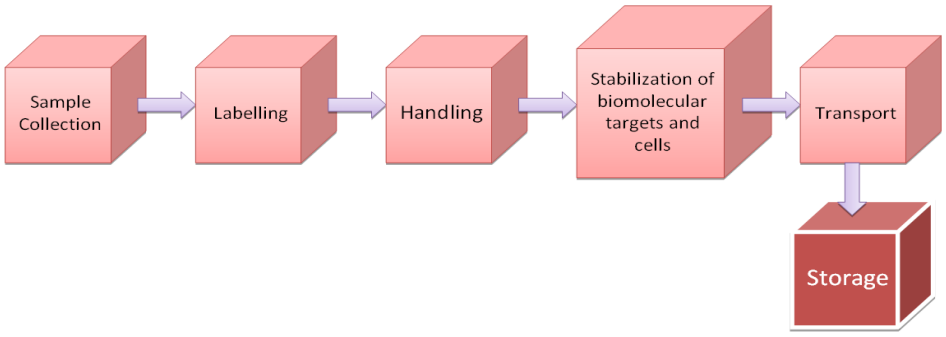

Figure 1. The preanalytical workflow for sample storage in biobanks. Samples may receive many different types of treatment, including different time delays between the steps. Differently treated samples may evolve differently and thus provide inconsistent subsequent analytical results.

The procedures currently adopted are mainly based on previous experience developed in classical diagnostics. Typically, they have never been validated by assessing the stability of the biomolecules to be detected in the downstream omics analyses. The preanalytical phase is the most vulnerable part of the overall testing process and is considered to be among the greatest challenges to the laboratory professionals. The scientific community is paying increasing attention to these issues also thanks to pioneering projects such as SPIDIA (http://www.spidia.eu/). The main outcome of this collaborative effort has been the identification of a number of critical steps in the preanalytical workflow that may influence the original molecular profiles of different biological specimens and the discovery that metabolites are the most affected molecules. This finding assigns a key role to metabolomics profiling as a tool to measure sample stability during and at the end of the preanalytical phase, as well as along the life time of samples in biobanks. Metabolites can change in a biospecimen for two main reasons: a residual enzymatic activity of the sample under study, which needs to be reduced by operating at lower temperatures and/or by using stabilizers, and a chemical

reactivity that may originate from multiple factors like exposure to light, air, temperature. Both mechanisms are potentially able to also affect the molecular profiles of other biomolecules. Therefore, the advantage of exploiting metabolomics to assess the quality of samples for and in biobanks is not limited to researchers intending to perform metabolomics profiling in the future but indeed provides an added value to all future omics analyses.

The above considerations have been developed in the context of various different activities, described in the following subsections.

3.2.2 Critical evaluation of NMR-based metabolomics

We have critically analyzed a number of previously published studies evaluating the effects of different treatments on urine samples onto metabolic profiles as determined by NMR. Furthermore, we addressed other aspects relevant to biobank activities aimed at the design of sample collections for metabolomics, providing indications on subject selection and NMR sample preparation. These evaluations have been systematically described in a review article [1]. The article is focused on the entire workflow of NMR-based metabolomics: from informed consent to sample retrieval from biobanks and additionally addresses some key variables that must be maintained under control during the analytical phase.

3.2.3 A European standard for pre-analytical processes

A specification document for pre-analytical processes for metabolomics in urine, serum and plasma has been produced as a follow-up of the SPIDIA project (http://www.spidia.eu/). This document is presently under examination at CEN (the European Committee for Standardization: Comité Européen de Normalisation) to be adopted as a European guideline, and is thus at the moment protected by confidentiality agreement. It provides operational directions on the key parameters that have to be controlled during the following steps: sample collection from the patient, handling and aliquoting, transport to and acceptance by the biobank or analytical lab, short and long term storage conditions, thawing before use

(compare to Figure 1). The document has been evaluated and discussed by the members of the committee CEN/TC 140/WG 3 “Quality management in the laboratory”. The members of EXCEMET (see point 3.2.6) will be invited to a CEN meeting at the end of this year for a critical reading before the production of the final document.

3.2.4 Metabolomics for the validation of standard operating procedures

As mentioned, the pre-analytical phase is considered to start from sample collection from the patient (Figure 1). In the case of intraoperative ischemia for tissue samples, medical procedures that precede sample collection were shown to have important effects on the resulting metabolic profiles (Cacciatore S et al. Effects of intra- and post-operative ischemia on the metabolic profile of clinical liver tissue specimens monitored by NMR. J Proteome Res. 2013, 12:5723-9). It is reasonable to think that similar caveats apply well beyond the specific case addressed. The role of medical procedures before sample collection is an aspect that is generally ignored in available standard operating procedures adopted by biobanks. Standardization of the pre-collection steps might not be always possible in a clinical context where operational procedures are dictated by patient care requirements. Nevertheless, the identification of the treatments that may induce meaningful changes in molecular profiles and the knowledge on how and to which extent they reflect in the downstream analyses are of vital importance for the researchers that will use the samples. A detailed annotation of all the procedures preceding sample collection and their inclusion in biobank databases together with the clinical information is an added value that can have a dramatic impact on the interpretation of the results of current and future studies.

The most obvious medical procedures that can alter the metabolome of biofluids derive from intake of specific molecules such as drugs or food supplements. As recently pointed out, the human metabolome consists not only of by-products of reactions catalyzed by enzymes encoded by the subject’s genome and by the genome of his/her bacterial microflora but has a non-negligible component due to molecules introduced via nutrition, pharmaceutical treatments and exposure to

pollutants (Wishart DS et al. HMDB 3.0—The Human Metabolome Database in 2013. Nucleic Acids Res. 2013, 41:D801-D807).

During the last year we have further analyzed the effects on the metabolic profiles of blood-derived samples due to common medical procedures, such as drug and probiotics administration. Unsurprisingly, when doses are above a certain concentration threshold, the signals of drugs become detectable in metabolomics profiles. The effects due to drug-induced alterations of the metabolism of the patient may be more subtle. Therefore, these aspects need to be considered and medical treatment annotated when designing cohorts and databases for biobank collections.

The effect of dietary restrictions instead has been studied in collaboration with the Medical University of Graz, using urine and saliva samples. Urinary profiles are dynamics and largely influenced by donor's lifestyle, but still contain an invariable part that is responsible of a strong individual signature, which is stable over the time scale of years. Saliva also contains a well-defined individual signature and appears to be less affected by the donor lifestyle. For both biofluids, the individual fingerprint is not significantly affected by dietary regimes [2].

3.2.5 Metabolomics for the control of samples in biobanks

As a further research activity to support the usefulness of metabolomics for biobanks, a design study aimed at monitoring the shelf-life time of samples stored in biobanks over the time scale of years has been implemented. The assessment of the degradability of human urine, serum and plasma samples during long-term storage is an issue of logistic and economic importance for biobanks, due to the high costs associate to controlled cryopreservation of samples. Because small molecule components of biofluids have been demonstrated to be sensitive reporters of degradation processes during the pre-analytical phase, we have evaluated the stability over time of the NMR-detectable part of the metabolome. The impact of storage at -80 °C over a time range of about 5 years was evaluated by monitoring the temporal deviations of ^{1}H NMR spectral profiles of multiple

aliquots of the same samples. Choosing aliquots from the same sample avoids possible confounding factors that might originate from interpersonal metabolome variability. To this purpose we have recorded the ^{1}H NMR profiles or aliquots of urine, serum and plasma samples at regular time intervals (from time 0, corresponding to the entry of the sample in the biobank, up to 4 years of storage). This permitted a comparative analysis of the ability of the available pre-analytical procedures to preserve the original metabolome on the long-term. Changes for the three most commonly used biofluids (urine, serum and plasma) were found to be essentially negligible whenever the adopted collection and handling procedures had followed up-to-date guidelines (Bernini et al Standard operating procedures for pre-analytical handling of blood and urine for metabolomic studies and biobanks, J Biomol NMR. 2011,49:231-43). Otherwise profile variations attributable to a large pool of metabolites were so important to severely hinder the recognition of the individual signature (Figure 2). This approach has been implemented at the da Vinci European Biobank in Florence, Italy as an internal quality assessment tool. Additionally, we are proposing this approach as a method to verify the performances of the single biobanks within the context of ring trials linked to BBMRI-ERIC.

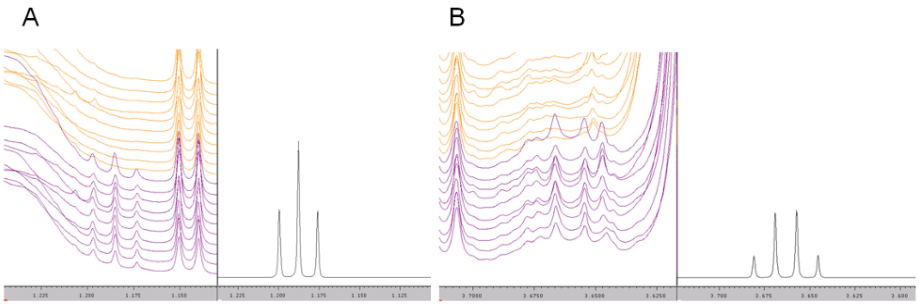

Figure 2. Variation of the metabolic profile of biobank samples over time. Orange traces: ^{1}H CPMG spectra acquired in 2009; Purple traces= ^{1}H CPMG spectra acquired in 2014. Black traces= spectrum of a pure ethanol solution. 10 NMR spectra of 10 plasma samples frozen at -80° C were acquired (purple) and compared to the spectra of the same samples acquired in 2009 before sample freezing (orange). It is possible to note the appearance of a triplet (A) and a quartet (B) due to the formation of ethanol, as confirmed by the comparison with a NMR spectrum of a solution of pure ethanol (black).

3.2.6 An Expert Center for Metabolomics, EXCEMET

Having identified the benefits of metabolomics approaches for sample quality evaluation, various COSMOS partners, as well as some other private and public stakeholders, have established a consortium of institutions involved in metabolomics activities. This is a follow-up of the implementation of a working group between the NMR metabolomics working group of COSMOS and BioMedBridges described in Deliverable 6.2 (available from the COSMOS website). The consortium, named EXCEMET (Expert Center for Metabolomics; http://www.excemet.org/) proposes itself as a reference infrastructure for biobanks. The synergy arising from coordinated actions between these scientific entities is foreseen as a method to push forward the metabolomics field and to provide a scientific support for the development of validated quality control measures based on molecular profiling within biobanks. EXCEMET has been established as a not-for-profit public-private-partnership based on a consortium agreement between participants from academia and industry. The involvement of the Medical University of Graz, with a research unit lead by Prof. Kurt Zatloukal, who coordinated the preparatory phase of the European biobanking and biomolecular research infrastructure (BBMRI) during the 7th EU framework programme, testifies the close links that have been established between the metabolomics and the biobanking community. EXCEMET has been described in a recent concept paper by the now established BBMRI-ERIC as a model of possible BBMRI-ERIC Expert Center [3].

3.3 Next steps

We will continue to monitor the scientific literature as well as to promote and develop initiatives to better circumstantiate the role of metabolomics in the validation of the procedures involving all the various steps of Figure 1, and beyond. Similar activities will be deployed to emphasize the value of metabolomics in monitoring the stability of samples stored in biobanks. In this latter respect, EXCEMET (see 3.2.6) will play a crucial role.

4 Publications

[1] Emwas et al. Standardizing the Experimental Conditions for Using Urine in NMR-based Metabolomic Studies with a Particular Focus on Diagnostic Studies: A Review, submitted

[2] Wallner-Liebmann et al. The impact of free or standardized lifestyle and urine sampling protocol on metabolome recognition accuracy, submitted

[3] van Ommen et al., BBMRI-ERIC as a resource for pharmaceutical and life science industries: the development of biobank-based Expert centers, European Journal of Human Genetics, in press

5 Delivery and schedule

The delivery is delayed: Yes ☑ No

6 Adjustments made

No

7 Efforts for this deliverable

Institute	Person-months (PM)		Period
	actual	estimated	
CIRMMP	7.83		
EMBL-EBI	0.5		
UOXF	1		
Total	9.33	2	

Appendices

None.

Background information

This deliverable relates to WP6; background information on this WP as originally indicated in the description of work (DoW) is included below.

WP6 Title: Coordination with BioMedBridges and biomedical ESFRI infrastructures
Lead: Claudio Luchinat
Participants: EBI-EMBL, LU-NMLC, CIRMMP, UOXF

This work package aims at maximizing communications with BioMedBridges and with its partner BMS infrastructures with an interest in metabolomics (Elixir, EU-Openscreen, BBMRI and Instruct), and helping steer the work of the other work packages to maximize the usefulness of the COSMOS activity for the current large scale EU biomedical infrastructures. The COSMOS consortium will actively participate in the concertation activities and meetings related with the e-Infrastructures area. We will help to optimise synergies between projects by providing input and receiving feedback from working groups addressing activities of common interest (e.g. from clusters and projects). If requested we will offer advice and guidance and be receptive for any information relating to 7th Framework programme implementation, standardisation, policy and regulatory, EU Member States initiatives or relevant international initiative.
Description of work and role

Work package number	WP6	**Start date or starting event:**		month 1				
Work package title	Coordination with BioMedBridges and biomedical ESFRI infrastructures							
Activity Type	COORD							
Participant number	1: EMBL/EBI	2: LU/NMC	10:CIRMMP	14: UOXF				
Person-months per participant	6	2	12	2				
Objectives								

1. maximizing communications with BMS infrastructures with an interest in metabolomics
2. helping steer the work of the other work packages to maximize the usefulness of the COSMOS activity for the current large scale EU biomedical infrastructures.

Description of work and role of participants

Task 1: Gather metabolomics requirements for BioMedBridges, BBMRI, ELIXIR and EU-OPENSCREEN CIRMMP will coordinate the gathering of requirements regarding the use of metabolomics data as a molecular phenotyping technique with the above-mentioned e-infrastructures. The University of Florence as a third party of CIRMMP will contribute to this task. EBI as coordinator of ELIXIR, BioMedBridges and responsible for database and standards development in EU – OPENSCREEN will contribute use cases from its on-going integration efforts. UOX is leading the development of the ISA infrastructure, which assists in the annotation, and local management of experimental metadata from high-throughput studies employing one or a combination of omics and other technologies, and will work toward integrating the findings from Task 1 in the ISA development.

Task 2: Coordinate with the activities of BioMedBridges regarding the standardization of metabolomics data WP 7 of the BioMedBridges grant will work in particular on NMR metabolomics data and towards a standardized description of sample donors, sample collection; pre-processing, analysis and evaluation will be established as a prerequisite for the inter-species comparison of metabolomics results. In this task all contributors to this task will ensure the appropriate coordination of the developments in WP2 of COSMOS and WP7 of BioMedBridges.

Task 3: Coordinate the activities of COSMOS versus the needs of Biobanks with respect to the association of NMR profiles to stored samples. The primary objective of biobanks is not merely archiving, but also distributing conserved and documented biological samples for research, and so they represent an irreplaceable support for all those studies in which the impact of the results is linked to the large number of the collected samples. The quality of stored biological samples is crucial for the outcome of subsequent studies. The molecules constituting the metabolic fingerprint are generally very sensitive to handling procedures and storage conditions, so metabolomics is a useful tool for checking and assessing the quality of stored samples. The NMR profile of a sample allows its evaluation in entrance (to decide its acceptance) and in exit (to decide if it is still good to be distributed), so it is important to associate each stored sample to the respective NMR metabolic profile. The aim of this task is to coordinate the activities of COSMOS, taking into account the requirements of Biobanks with respect to the association of NMR metabolic profiles to stored samples. BBMRI (Biobanking and Biomolecular Resources Research Infrastructure) was one of the first European Research Infrastructure projects funded by the European Commission (EC). The EC-funded preparatory phase of BBMRI came to its end in January 2011. During the past 3 years BBMRI has grown into a 53-member consortium with over 280 associated organisations (largely biobanks) from over 30 countries, making it the largest research infrastructure project in Europe (http://www.bbmri.eu/). In this task we will

interface with BBMRI and develop a strategy for the use of Metabolomics for Biobank sample monitoring and deposition of the sample status data in COSMOS partner databases.

Deliverables

No.	Name	Due month
D6.1	Document describing requirements for relevant biomedical infrastructures with regard to Metabolomics	6
D6.2	Establishment of an NMR metabolomics working group between COSMOS and BioMedBridges	12
D6.3	Joint consensus document between COSMOS and BioMedBridges	18
D6.4	Joint consensus document between COSMOS and BioMedBridges (Updated)	36
D6.5	Report on the recommendations of the use of Metabolomics of Biobank sample monitoring	24