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Review Article

Biobanks and Clinical Research: An "Interesting" Connection

Abstract

In our era, biobanks ensure preservation of specimens' quality in short or long time storage. For each type of material and for each kind of organism, there is a specific preservation protocol. Actually, the efforts of single scientists or Institutions are not sufficient for research, especially in rare diseases field. The building of network that join together biobanks, research institutes, universities, pharmaceutical companies and patients' associations answers to research's needs. The creation of national and international networks had greatly contributed to share samples and their related information. In this review, we describe the European situation and how different associations and Institutions are joined together in wide networks. We marked the efforts and the ways needed to link and to harmonize Institutions placed in different countries and subjected to different national laws in lacks of unified legislation. We reviewed primary, biobank needs and principal preservation technics and protocols. Our observations had marked the importance of building wide networks. We would concluded noting the importance to extend actual networks including other national or foreign institutions. The networks should be organized to provide flexibility for facilitating its growth.

Abbreviations

RD: Rare Disease; EBB: Euro Bio Bank; TREAT-NMD: Translational Research Europe: Assessment and Treatment of rare inherited Neuro Muscular Diseases; BBMRI-ERIC: Bio Banking and Bio Molecular Resources Research Infrastructure-European Research Infrastructure Consortium; SOP: Standard Operating Procedure; ELSI: Ethical, Legal and Social Implication; OECD: Organization for Economic Co-operation and Development; IRDiRC: International Rare Diseases Research Consortium; TNGB: Telethon Network of Genetic Bio banks; UNIAMO FIMR: UNIAMO Federazione Italiana Malattie Rare; IARC/WHO: World Health Organization's International Agency for Research on Cancer; EURORDIS: European Organisation for Rare Diseases; ERN: European Reference Network; EUCERD: European Committee of Experts on Rare Diseases; AFM: Association Française contre les Myopathies; UDBN: UK DNA Banking Network; CPA: Cryo Preservative Agent; PBMC: Peripheral Blood Mononuclear Cell.

Introduction

The modern biology has undergone a profound transformation becoming an "information science". Actually, biology needs to apply to databases well structured, continuously upgraded, massively enriched at exponential rate and freely accessible. In order to build these databases, the efforts of individuals or small Institutions are not sufficient, biology needs institutional collaboration and geographically distributed efforts. The collaboration requires mutually agreed policies, standard operating procedures for sample collection, and common standards for information's representation and sharing [1].

What is biobank?

The term "biobank" refers to a biological specimens' collection like DNA, RNA, tissues and cells. The samples are linked, in an informatics network, to several information about donor like biological, clinical and epidemiological features or about his lifestyle. The samples could be donated by healthy volunteers or people affected by some disease. Some biobanks had the availability of clinical follow-up of patients [2]. In a biobank, you could collect samples of human beings, animals, plants or microorganisms; there are no limit for sample time storage. The specimens could be used for studying in general or disease-specific [3]. Every year millions of samples are stored in biobanks for diagnostic or research purpose.

European networks

Millions of samples are collected in biobanks across the Europe. Although individual collections could be organized and accessible, the resources are subjected to fragmentation, insecurity of funding and incompleteness [4]. In this context, several efforts have been made to create some European networks connecting all biobanks one to another in order to harmonize them.

For example, the need to have a wide network to exchange samples and information is very strong in the field of rare diseases (RDs). In RDs field, there are few patients and samples for each disease. Sharing materials and data is essential for identifying disease-causing genes, studying pathological mechanisms and developing treatment. In order to gain these aims, in 2001, the EuroBioBank (EBB) was established involving 16 partners of eight European countries (Belgium, France, Germany, Hungary, Italy, Malta, Slovenia and Spain). In Europe, EBB (www.eurobiobank.org) was the first biobanks network operating to collect DNA, cells and tissues for research in RDs. EBB network aims to identify and locate repositories, harmonize and disseminate quality banking practices, distribute quality materials and data and spread knowledge through training courses, conferences, articles and website [5]. In 2007, EBB became part of TREAT-NMD (partner of Fondazione Telethon) and in 2012 joined Fondazione Telethon. Actually, EBB network are composed by 25 members of which 21 are biobanks and 4 are not biobanks; some of which are non-European

Table 1: EBB network members.

Member	Short name	Type of association	Stored materials	Country
European Organization for Rare Diseases	EURORDIS	Patients' association		Europe
Association Française contre les Myopathies	AFM	Patients' association		France
Istituto Nazionale Neurologico Carlo Besta	NEUMD-INNCB	Research institute		Italy
Fundación para la Cooperación y Salud International Carlos III—ISCIII	Fundación CSAI Carlos III—ISCIII	Research institute Biobank	DNA, plasma, serum and lymphocyte cells from each donor	Spain
Généthon III	Généthon	Biotherapy R&D organization Biobank	Blood, DNA and lymphocyte of peoples affected by genetic (mainly neuromuscular) disease	France
Centre de Génétique Humaine UCL	LOUVAIN	University		Belgium
University of Ljubljana, Medical Faculty	University of Ljubljana	University Biobank	Biomaterial from autopsies - including foetal biomaterial - of patients affected by neuromuscular diseases	Slovenia
The University of Malta	UOM	University Biobank	DNA from patients with rare disease	Malta
Muscle Tissue Culture Collection at the Friedrich-Baur-Institut of the Ludwig-Maximilians-Universität, Munich	MTCC	Biobank	Myoblasts of a wide range of neuromuscular diseases	Germany
Second University of Naples	SUN	University Biobank	DNA and tissues from patients and their families, affected by different neuromuscular disorders (primary cardiomyopathies)	Italy
Fodor József national Center for Public Health, Budapest	NCPH	Biobank	DNA	Hungary
Ospedale Maggiore Policlinico IRCCS, University of Milan	NMUNIT—UNIMIOM	University Biobank	Skeletal and cardiac muscles, peripheral nerve, DNA samples and cell cultures from people affected by neuromuscular disorder	Italy
University of Padova, Department of Neurological and Psychiatric Sciences	NMTB	University Biobank	Muscle tissues to a wide range of neuromuscular disease	Italy
Bio Expertise Technologies	B.E.T.			
Université Joseph Fourier—Grenoble 1	UJF	University		France
TEAMLOG SA	TEAMLOG			
Bank for the Diagnosis and Research of Movement Disorders, Istituto Neurologico Carlo Besta	MDB-INNCB	Biobank	Muscle tissue, DNA and muscle cells from patients with neuromuscular diseases	Italy
Bank of the National Laboratory for the Genetics of Israeli Populations	NLGIP	Biobank	B-lymphoblastoid cell lines and DNA of Jewish and Arab ethnic groups in Israel	Israel
MRC Centre for Neuromuscular Diseases BioBank, London	CNMD-BBL	Research institute Biobank	Myoblast and fibroblast samples from people affected by congenital muscular dystrophies or congenital myopathies	UK
MRC Centre for Neuromuscular Diseases BioBank, Newcastle	CNMD-BBN	Research institute Biobank	Myoblasts, fibroblasts, plasma and serum of patients with neuromuscular disorder	UK
Quebec Myotonic Dystrophy Biocatalog	QMDB	Biobank	DNA, tissues and cells	Canada
Cell line and DNA Biobank from patients affected by Genetic Diseases, Istituto Giannina Gaslini, Genova	IGG-GB	Biobank	Dermal fibroblasts, lymphoblasts, amniocytes, chorionic villous cells and RNAs/DNAs derived from patients affected by more than 250 different genetic defects	Italy
Galliera Genetic Bank, Ospedali Galliera, Genova	GGB	Biobank	Samples from families with subjects affected by rare genetic disorders	Italy
Cell lines and DNA Bank of Rett syndrome, X-linked mental retardation and other genetic diseases, University of Siena	biobankUNISI	University Biobank	DNA and cell lines (lymphoblastoid cell lines, leukocytes or fibroblasts) from patients	Italy
Parkinson Institute Biobank, Istituti Clinici di Perfezionamento, Milano	BPI	Biobank	DNA, RNA, Serum, Fibroblast cell lines and Post-mortem brain tissues	Italy
Genomic Disorders Biobank IRCCS Casa Sollievo della Sofferenza, S Giovanni Rotondo	GGDB	Biobank	DNA, RNA and tissue cell lines from individual affected by genomic and genetic disorders and their relatives	Italy
Fondazione Telethon	FTELE	Non-profit organisation for research		Italy
Dipartimento Ligure di Genetica, Genova	DLG			Italy

Abbreviations: ISCIII: Instituto de Salud Carlos III; UCL: Université Catholique Louvain; IRCCS: Istituto di ricovero e cura a carattere scientifico; MRC Medical Research Council.

biobanks but are placed in Canada and Israel [5] (Table 1). As part of TREAT-NMD, EBB collaborated with BBMRI-ERIC. In this network, all biobanks in membership had the custodian of samples and EBB are a virtual biobank in which easily find desired sample for research [5]. All biobanks in the world are encouraged to join to EBB Network. For join to network is sufficient to adhere to minimum entry criteria [5]:

- Presence of collections of RD biological samples and their availability to the scientific community;
- A quality-control system for the management of the biobank;
- Standard operating procedures (SOPs) regulating sample and data acquisition and sample processing, storage, and distribution.

The candidate biobanks should also adhere to Ethical, Legal and Social Implication (ELSI) principles and comply with the recommendations issued by the Oviedo Convention and the OECD Task Force on Biological Resource Centers and with the national and European laws and regulations [5]. In order to create an EBB virtual catalogue, all partners agree the following data set [5]:

- Type of sample;
- Classification of the disease based on ICD-10 identifier and name;
- MIM number and name;
- Number of families;
- Number of patients;
- Anatomic origin;
- Biobank contact;
- ORPHA code.

In the EBB catalogue (http://www.eurobiobank.org/en/services/CatalogueHome.html) you can search for biological samples by type of biological material and disease [5]. In order to help pharmaceutic industries to discover disease biomarkers, new therapeutic approaches or new drugs, EBB Network has been involved in several pharmaceutic projects [5]. EBB brought together several funding organizations, governments, academies, industries and patients' organizations that share common goals and principles into the International Rare Diseases Research Consortium (IRDiRC, http://www.irdirc.org).

IRDiRC aims to have a diagnostic test for most rare diseases and 200 new therapies by 2020 [6]. In 2012, European Union's Seventh Framework Programme under IRDiRC founded RD-Connect. RD-Connect is a global infrastructure that joins genomic data with registries, biobanks, and clinical bioinformatics tools [7]. It aims to develop:

Table 2: TREAT-NMD's partners.

Partner	Type of association	Country
University of Newcastle upon Tyne	University	UK
Institut National de la Santé et de la Recherche Médicale	National institute of research	France
Leiden University Medical Center	University	The Netherlands
Muskeldystrophie-Netzwerk	Clinicians and scientists association for research in muscular dystrophy	Germany
European Neuromuscular Centre	Consortium of patients' organizations	The Netherlands
Summit Therapeutics	Drug discovery company	UK
Association Française contre les Myopathies and Institut de Myologie	Patients' association	France
Biozentrum, University of Basel	University	Switzerland
European Organisation for Rare Diseases	Patients' association	Europe
Karolinska Institute	University	Sweden
King's College London	University	UK
Santhera Pharmaceuticals	Pharmaceutical company	Switzerland
Helsingin yliopisto	University	Finland
Medical Research Council	Research councils	UK
Fondazione Telethon	Non-profit organisation for research	Italy
Université Catholique de Louvain	University	Belgium
Universitat Autònoma de Barcelona	University	Spain
GenoSafe	Contract research and consulting organization	France
ACIES		France
National Institute of Environmental Health	Publicly-funded institute for research and divulgation	Hungary
Genethon	Biotherapy R&D organization Biobank	France
University College London	University	UK

- an integrated platform to host and analyze genomic and clinical data from research projects;
- Clinical bioinformatics tools for analysis and integration of molecular and clinical data to discover new disease genes, pathways, and therapeutic targets;
- Common infrastructures and data elements for rare disease patient registries;
- Common standards and catalogue for rare disease biobanks;
- Best ethical practices and a proposal for a regulatory framework for linking medical and personal data related to rare disease [7].

TREAT-NMD (www.treat-nmd.eu) is an excellent network funded by the European Commission (framework programme 6) which aims to provide an infrastructure to support all stages of therapy development, and to promote necessary collaborations among patients, advocacy groups, academic Institutions, industries, and governmental agencies. TREAT-NMD was created by the efforts to address the fragmentation currently hindering translational research for therapies in rare neuromuscular diseases (NMD). In order to gain its aims, TREAT-NMD brings together experts from different European centers [8] (Table 2). The centres work together in order to accelerate clinical application of promised treatments. Noting differences in care implementation, this project spreads standards and best practises of care via website to harmonize the access to expert practises for patients across Europe [8]. Another infrastructure of TREAT-NMD is a global registry of patients of more than 20 different country. The patients' registry is very important to facilitate and accelerate clinical research and clinical trials and to give patients improved access to relevant information on standards of diagnosis and care. In fact, in some areas, the specific mutation will determine the applicability of a particular therapeutic technique [8].

The best solution to the demand for well-annotated and properly preserved specimens, in the field of genetic diseases, is the building of a bio banks network: Telethon Network of Genetic Biobanks (TNGB). TNGB (http://biobanknetwork.telethon.it/) is an Italian association of repositories created with the aim to generate a unique catalogue that actually lists 750 genetic diseases [9]. The network is composed by 10 biobanks:

- Cell Line and DNA Biobank from patients affected by Genetic Diseases (Genoa);
- Galliera Genetic Bank (Genoa);
- Parkinson Institute Biobank (Milan);
- Cell line and DNA bank of Rett syndrome, X-linked mental retardation and other genetic disease (Siena);
- Neuromuscular Bank of Tissues and DNA samples (Padua);
- Bank of DNA, Cell lines and Nerve-Muscle-Cardiac tissues (Milan);
- Cell, tissues and DNA from patients with Neuromuscular Diseases (Milan);
- Genomic Disorder Biobank (S. Giovanni Rotondo);
- Naples Human Mutation Gene Biobank (Naples);
- Cell Line and DNA Biobank of Paediatric Movement Disorders (Milan) [9].

Every network's biobank is established in Institutions with longstanding tradition and internationally expertise in diagnosis and research. TNGB is sustained by collaboration with skilled clinicians, pathologists, biochemists and geneticist [9]. The network's aims were to centralize very rare samples and data, minimize biases potentially arising from heterogeneity in the quality of samples by developing standard procedures and common quality assurance policies, increase collaboration inside the biomedical community and promote collaboration with patients' associations. Building a powered infrastructure and defining network's ethical, legal and societal policies and standard operating procedures, TNGB had be able to harmonize pre-existing biobanks collection and data annotation [9]. Actually, TNGB collects about 76,000 biospecimens from 750 genetic defects included in cardiovascular disorders, chromosome aberrations, craniofacial disorders, deafness; dermatologic disorders, endocrine disorders, genomic disorders, hematological diseases, intellectual disability, x-linked intellectual disability, metabolic disorders, neuromuscular disorders, neurologic disorders, movement disorders, ophthalmologic disorders, primary cardiomyopathies, rare tumors, renal disorders, Rett syndrome, skeletal dysplasia and white matter disorders. The biospecimens mainly stored in TNGS' biobanks include fetal and adult cell lines (amniocytes, trophoblast cells, fibroblasts, myoblasts, lymphoblasts and T-lymphocytes activated with interleukina-2), peripheral blood lymphocytes muscle and nerve tissues, tissues derived from fetal loss, DNA/RNA samples, serum/plasma and whole blood samples and iPS cells. Each sample is managed by local biobank but it have to include at least the minimum TNGB-shared data set [9]. The data set are composed by:

- Donor'/patient's generalities (name, date of birth, address, ethnic origin, gender);
- Phenotype (affected/not affected);
- Essential anamnestic data (presence of consanguinity and/or familiarity, tissue and/or organ anomalies, laboratory test anomalies, etc…);
- Diagnosis data (modality, center performing diagnosis);
- Sample data (code, type, data of collection, etc…) [9].

A close relationship with patients' associations (UNIAMO FIMR) is very important to improve TNGB infrastructure. The involvement of patients and their families has proved to be fundamental tool to gain a critical mass of samples, that is essential for research into very rare diseases [9,10] and to ensure that patients' needs and expectations are taken into due consideration [9].

BBMRI-ERIC (www.bbmri-eric.eu) was built on existing sample collections [4], it had created a network linking several biobank in Europe [1]. In BBMRI Sixteen Member States and one International Organisation are joined together building a pan-European distributed research infrastructure of *biobanks* and *biomolecular*

resources. This European organization aims to improve accessibility and interoperability between academic and industrial parties to benefit personalized medicine and diseases prevention, to promote development of new diagnostics devices and medicines. BBMRI-ERIC sustains the partnership between public and private expert centers designing a new structure that would face actually difficulties [11].

BBMRI provides:

- Biobanks of different formats (based on collections of DNA, tissue, cells, blood and other body fluids, together with pertinent medical, environmental, life-style and follow-up data);
- Population cohorts;
- Clinical case/control cohorts including disease-focused cohorts;
- Biomolecular resources (comprising antibody and affinity binder collections, ORF (Open Reading Frame) clone collections, siRNA (small interference RNA) libraries, proteins, cellular resources, etc.);
- Enabling technologies, high-throughput analysis platforms and molecular tools to probe gene, protein and metabolite activities;
- Harmonized standards for sample management;
- Harmonized databases and biocomputing infrastructure;
- Ethical, legal and societal guidance and platform [4].

BBMRI gives European scientists and industry some advantages:

- An unified catalogue information on biological samples and collected data;
- Improved reliability and reduced ambiguity in comparing and interpreting results;
- Aa setting to establish an open-source based federated database structure that can guarantee the same standard of data quality in annotation, while protecting donors' privacy;
- Access to a Europe-wide data and sample set, thus providing data with better statistical power or permitting the investigation of rare or highly diverse diseases;
- Capacity to develop prospective collections meeting the needs of particular research projects or clinical trials;
- Compliance with ethical and legal requirements.
- Sound governance system building on input by all stakeholders [4].

The members of BBMRI-ERIC are Kingdom of Belgium, Czech Republic, Federal Republic of Germany, United Kingdom of Great Britain and Northern Ireland, Republic of Estonia, Hellenic Republic, French Republic, Italian Republic, Republic of Malta, and Kingdom of the Netherlands, Republic of Austria, Republic of Finland and Kingdom of Sweden. Switzerland, Norway, Poland, Turkey and IARC/WHO (The World Health Organization's International Agency for Research on Cancer) are observers [11]. BBMRI-ERIC is organized in national nodes, each of which coordinates the national biobanks and biomolecular resource and links them activity into a pan-European mechanism. This infrastructure is organized in way to provide flexibility for facilitating growth of the network. Membership is non-exclusive but favors members that can link BBMRI to other national, European or global initiatives [4].

Actually, in BBMRI-ERIC network, 515 biobanks of different states members are joined together. The biobanks are classified for type: clinical, population, research study, non-human, and standalone collection. In BBMRI-ERIC's website, you can search by material type, diagnosis available, country, biobank size, type of biobank and type of collection. The website well describes biobanks European distribution. In Austria, there are 4 biobanks of which 3 are clinical and 2 are non-human. In Germany 10 biobanks are joined in the BBMRI-ERIC network, all biobanks are clinical and only one is population. In the network, there are 5 Czech Republic's biobanks, 89 France ones, a Greek one and a Maltese one, all these biobanks are clinical. In Estonia there are 7 biobanks, of which 1 is research study, 2 are population and 1 is clinical. In the network, there are 8 Finland biobanks, of which 4 are clinical and 1 is population. In Netherlands, there are 189 biobanks joined in BBMRI-ERIC network, of which 3 are clinical, 34 are population, 152 are research study and 189 are standalone collection. In Norway, there is only one population biobank. 69 Italian biobanks are linked in the network, of which 6 are clinical. 114 Sweden biobanks are joined in network; all these biobanks are standalone collection. At last, there are 13 Belgium and 4 Poland biobanks.

The relationship between biobanks network and patients' associations is significant. An important European patients' organization active in the field of RDs is EURORDIS (European Organization for Rare Diseases, www.eurordis.org). Into EURORDIS, there are patients affected by more than 4,000 RDs living in 58 different countries. Since 2004, EURORDIS participated to several discussions on shaping Centers of Expertise and European Reference Networks (ERNs) in order to improve the access to appropriate diagnosis and cure for people affected by RDs. This organization delivers the expectations of patients and their families regarding the organizations for their care. EURORDIS contributes to the Recommendation of the European Committee of Experts on Rare Diseases (EUCERD) on the "quality criteria for Centers of Expertise for Rare Diseases in Member States". In this context, ERNs for rare diseases could understand how they should function based on patients' life experience [12]. An Italian association of patients is UNIAMO (http://www.uniamo.org/) that is part of EURORDIS. UNIAMO's mission is to improve life's quality of people who are affected by rare disease. This organization aims to gain its objectives promoting respect of patients' right. The Association Française contre les Myopathies (AFM, http://www.afm-telethon.fr/) is a French association of patients. In the long term, it aims to find a therapeutic solution to neuromuscular diseases through medical research and more specifically research on gene therapies. In the short term, the AFM aims to improve the lives of patients through better access to care, and better social support. Initially devoted solely

neuromuscular diseases, the research projects supported by the AFM now cover a wide range of genetic diseases.

Biobank's needs to ensure high quality

A biobank needs adequate resources in terms of personnel, space, laboratories, instrumentation, computers and quality system.

To ensure the specimens high quality, biobank needs qualified and trained personnel to adhere to SOPs and periodic update training [13]. To ensure continuous biobanking service, two or three laboratory technicians are required at minimum [14].

In biorepository, a director who is familiar with both clinical and research aspect of translational research should be appointed. The director prior responsibility is to ensure communication and collaboration between laboratory researchers and clinicians. An experienced research manager should be appoint to direct daily activities. He should be familiar with human-tissue handling, biosafety, shipping regulations, and site-specific health and safety issues; he also might collect, process, and ship specimens, maintain the database, order supplies, update the budget and revise the SOPs. To optimize the research environment, the research manager should frequently interact with investigators [15].

The ideally biorepository is located within academic hospital, clinical and research support. The space must to be approved to level-2 biosafety; it needs directional airflow or use of biological safety cabinet. Only designed staff could enter in biobank. To manage samples with potentially lethal infectious agent, the required space must to be approved to level-3 biosafety. Principal equipment required includes a lockable, alarmed freezer (-70°C or lower), a refrigerated centrifuge with sealed buckets, a refrigerator-freezer and computer equipment [15]. The laboratory can share existing resources like wet or dry ice, biological safety cabinets, and autoclaves with the hospital or Institution if the risk of cross-contamination is low. Other supplies are specific for the type of collected tissue. In small biorepositories, the samples could be stored in cryovials with high-quality, laboratory printer–generated labels; contrarily in large biorepositories, two-dimensional barcode system is needed to ensure automate storage reducing human error and increasing efficiency. For facilitating the transfer of samples to laboratories without barcode scanner, human-readable labels should be used on tube [14,15].

In small and medium biobanks, a software package may be use satisfactory, but a more extended structure ideally should use an own dedicated software. In this case, in order to modify and improve the software to satisfy laboratory's needs, a computer engineer or computer programmer is required [14].

Long-term storage technics

The facilities, personnel and expertise to the maintenance of biobanking have high costs. Process and scientific imperatives would be gained with centralized systems of recruitment, date collection, sample processing and follow-up. The centralized approach ensures increased efficiency promoting standardization. Opposite, distributed approach needs to establish and maintain dozens or hundreds centers. In order to guarantee the same high-quality level, each center must become expert in all study aspects [16].

For example, UK DNA Banking Network (UDBN) built a network in which it aggregates and manages samples and data originated by others. The network comprises, on one hand investigator groups led by clinicians each with a distinct disease specialism and on the other hand, a research infrastructure to manage samples and data. Reproducibility and quality level in entire network are ensured by standardization of all operating procedures and by monitoring of all procedures. This structure needs coordination and continuous dialog between the investigators groups and the research infrastructure. The process of integration is facilitated by biannual network meeting. This approach reduced the need for repeated investments in new infrastructures and resource management. It help clinicians to focus on their core competence in study design [17].

Diagnostic staff and researcher's mast have first well thought out and designed the study to follow in order to have a well-organized collection and good protocols for its management. After study conclusion, most of the time, many questions arise; to answering them, stored samples need to be analyzed. Sometime the samples are analyzed to test new technologies. For these reasons, the samples should be preserved in good condition for a long time. In order to satisfy these researchers' needs, a sample can be divided in several different vials or different materials of the same sample can be divided in different vials. Automatic procedures help to quality control and decrease costs [14].

In order to preserve specimens' quality, the first step to set up a biobank is to define guidelines for collect samples and evaluate their quality. Appropriate management procedures ensure correct identification of samples and maximize the recovery of material. Usually viability, pathogenicity, morphological or physiological parameters and genomic stability of cells or strains after thawing may be compared to the same parameters before cryopreservation [14]. The factors known which influence samples stability include:

- The use of preservants or anticoagulants;
- The temperature range during the time between sample collection and processing and during storage;
- The time spent between initial processing and storage;
- The sterility during sample collection and processing;
- The presence, in the sample, of active endogenous degrading or inhibiting substances [14].

Specific conditions are requested to store human, animal and microbial specimens or separated cellular component. Cryopreservation technics enable to preserve biological material life at sub-zero temperatures preventing biochemical reactions. Actually, cryopreservation methods involve slow/rapid freezing or vitrifying of cells in cryopreservative agent (CPA) presence. The cells exposure to low temperatures could cause severe damages to them. Lethal cryoinjuries depend on intracellular ice formation, on the efflux of water outside the cell and on an increase in the concentration of intracellular salts in the solution. The temperature range of +10°C-0°C is the most critical for cell damages, differently below 0°C minor damages occur. The cryopreservatives could protect cells by binding

intracellular water, by preventing ice crystals formation and excessive cellular dehydration or by reducing intracellular salts concentration [14]. The CPAs are divided in two classes:

I. Intracellular CPAs: molecules like dimethyl sulfoxide (DMSO or Me_2SO), glycerol, and 1, 2-propanediol can penetrate cell membrane;

II. Eglycol (PEG) cannot penetrate cell membrane.

In order to prevent cell lysis, differentiation or toxicity, the CPAs addition and removal must be controlled [18].

In slow freezing, tissues or cells are cooled down at rate of 1°C/min and stored at -80°C [18,19]. In similar conditions, the water has time to diffuse to extracellular solution achieving a new equilibrium point state. Because of the low rate of cooling down, the cells are exposed a longer time to high CPA concentration [18,20]. During slow freezing, empty channels remain between ice crystals; cells squeeze into them. As temperature decreases, the ice crystals grow to close the channels exerting mechanical forces on cell and causing damages in them [18,21,22]. In rapid freezing, tissues or cells are cooled down at rate of 60-120°C/min [18,23]. In similar condition, the forming ice in extracellular region removes water from solution increasing CPA solution concentration. At high cooling rate, water does not have enough time to diffuse to extracellular region causing the formation of intracellular ice crystals. Intracellular ice crystals damage cells compromising viability and functions of cells [18,24].

Vitrification minimizes cryoinjury ultra-fast cooling cells. The cells are submerged in liquid nitrogen (-196°C) or in vapor nitrogen (-165°C) transforming them into a glass-like solidification status. This method avoids ice crystals formation [18,25,26]. Unfortunately, the high CPA concentration, that is required to achieve vitrification, results in osmotic cells dehydration [18]. Different vitrification approaches reduce cryoinjuries enhancing the cooling/rewarming rate up to 700,000°C/min and reducing the CPA concentration required [18,23,27-29].

Freeze-drying (lyophilization) is used to improve the long-term storage stability of unsTable materials into solid form by removing the solvent (usually water). This procedure consists of some different steps. At first, the vials containing solution are cooled down at temperature of -40°C (in several steps) in which most of the water is transformed into ice. Generally, the solute remains amorphous in the freeze concentrated state and the 20% of the unfrozen water is in the amorphous phase [30,31]. Subsequently, a vacuum pump maintains the chamber pressure between 50 and 200 mTorr (below the vapor pressure of ice at the target product temperature). This pressure conditions ensure a high sublimation rate. Next, the temperature is raised to facilitate sublimation of ice. During this phase, the temperature is maintained below the maximum allowable product temperature. The most of unfrozen water is removed in last step by desorption at elevated temperature with the chamber still under vacuum. At the end of the entire process, only 1% of water remains in samples [30,32].

How to preserve the specimens?

As already said, you may store into a biobank tissues or cells deriving from several kind of organisms like human beings, animals, bacteria, fungi, parasites or viruses, or different type of materials like serum, plasma, DNA or RNA.

Human or animal cells or cell lines may be collected for several reasons. Principal problems about these cell types depend on the different reaction to freezing conditions and on the existence of cell-to-cell contacts or mutual relationships. The human or animal cell types most frequently stored are the PBMCs (Peripheral Blood Mononuclear Cells). The PBMCs can be isolated, from citrated blood sample, by density gradient centrifugation over lymphocyte separation medium. The PBMC layer is harvested and washed with PBS. The red blood cells contained in it are lysed using Pharm Lyse by incubating 2×10^8 cells in 20 ml of 1/10 diluted Pharm Lyse in distilled water. After 30 minutes of incubation in the dark, the reaction has to be stopped by adding 30 ml of PBS with 1% FBS [33]. Subsequently, isolated PBMCs have to be suspended at concentration of circa $1x10^7$ cells/ml and cryopreserved in cryopreservation solution like GHRC-CryoMedium I, GHRC-CryoMedium III, IBMT-Medium I or IBMT-Medium II [33]. Aliquots of 1ml PBMCs suspension must be put in pre-cooled (-20°C) cryovials. Finally, prior to transfer cells into nitrogen (gas or liquid phase), in order to allow a correct freezing of them from +4°C to -80°C, the cryovials have to be placed in freezing container for freezing at -80°C (cooling rate of 1°C/min) [33-35]. For thawing the samples, the cooled cryovials are transferred from nitrogen to water bath at temperature of 37°C. The samples stay into bath until only little ice remains. Thawing medium (IMDM medium containing L-glutamine, 25 mM HEPES buffer, and 3.024 g/l sodium bicarbonate supplemented with 10% of heat-inactivated FBS) has to be added to PBMC suspension. The tubes containing PBMCs added with thawing medium are centrifuged with 400g for 5 minutes. Finally PBMCs are suspended in thawing medium [33]. In biological and clinical studies, serum and plasma are most common materials used. Plasma is liquid part of unclotted blood; it is separated by centrifugation of blood containing an anticoagulant. Differently, serum is separated by centrifugation of blood without anticoagulant. These two sample materials are used to evaluate the person's state of health or disease and to define the interactions between drugs and host cells. The application of standardized procedures of separation within two hours from sample collection ensure comparable values of analytes between serum and plasma. Contrarily, red blood cells' lysis causes significant differences in levels of potassium, phosphorus, albumin, and lactate dehydrogenase [36,37]. In order to prevent ongoing metabolism as well as hemolysis and analysts' leakage between the plasma and serum and cellular compartments, for biochemical analysis the samples must be separated as quickly as possible [37,38]. It is known that freeze-drying increase maximum storage time and the number of times to brought samples to room temperature. However, the stability of biomolecules is ensured adequately by -80°C or liquid nitrogen storing. To prevent degradation of proteins and nucleic acids, repeated freeze/thaw cycles may be avoided. Recently, a study verified stability of 159 metabolites (except for methionine sulfoxide) to two freeze-thaw cycles [37,39,40]. However, the ideal conditions for long time storing of serum and plasma remains to determine [37].

Whole blood and blood cells represent the main source of

nucleic acids [37,41]. Red blood cells, white blood cells and platelets suspended in plasma, compose whole blood. Whole blood is collected with anticoagulant. The anticoagulated part of blood sample composed by white blood cells and platelets is named buffy coat. The biobanks, in which store DNA or RNA, are required for functional genomic analysis, exploration of copy-number variations (CNVs) or chromosomal regions in association with non-Mendelian diseases, epigenetic or transcriptomic studies [37]. EDTA, citrate and heparin are the anticoagulants most frequently used in storage. EDTA may be used for some types of studies, citrate is more appropriate in cultures of white blood cells, but heparin is not recommended because it inhibits PCR reactions [37,42,43]. Frozen causes nonviable injury in stored cells. The DMSO is able to preserve blood cells viability. If DNA cannot be extracted immediately, whole blood have to be stored at temperature of -80°C. Temperature of -80°C ensures specimens stability for years [37,42,43]. The buffy coat for RNA studies have to be preserved at temperature of -150°C to ensure RNA stability. Differently, buffy coat for DNA studies may be stored at -80°C, without impact extracted DNA quality [37,43-45]. RNA is the most insTable molecule; to preserve RNA in intact state for more than 50 months, it must be stored in liquid nitrogen at temperature of -140°C to inactivate biochemical reactions [37,46-48]. However, -130°C is suggested by WHO-IARC biobank as optimal temperature for RNA preservation [37,49]. In order to simplify and to ensure major safety of storage, several centres use to maintain samples at -80°C frozen temperature [37,44,50]. Recently, some studies marked that extracted RNA value is not affected by -80°C temperature preservation [37,51]. The use RNase inhibitors could preserve RNA quality [37]. Contrarily, DNA is characterized by major stability. Extracted DNA quality could be preserved by storing for numerous weeks at 4°C or for months at temperature of -20°C [37,44,52]. However, -80°C temperature storage in aqueous buffer or in nuclease free water ensures major stability to nucleic acid [37].

In several laboratories, different microorganisms are collected for epidemiological studies, quality controls, teaching and research. In order to maintain microorganisms in appropriate conditions for the above-cited needs, cryopreservation represents the best practice [53]. In order to reduce the risk of cryoinjuries, to control the cooling rate (-1°C/min) and the presence of a cryoprotectant (glycerol, trehalose or DMSO) are essential [53,54]. Cryoinjuries derive from several kinds of stresses that include concentration effects caused by pH changes, precipitation of buffers, dissolved gases, electrolytes concentration, intracellular crystallization resulting from loss of the water of hydration from macromolecules, cell shrinkage [53,55,56] and ice damage. Some microorganisms named "preservation recalcitrant", such as *Helicobacter* (bacteria), *Pythium* and *Saprolegnia* spp (fungi), are prone to cryoinjuries and exhibit poor viability after towing. The preservation of recalcitrant microorganisms needs specific designed protocols. Bacteria may be cryopreserved by bead system. Bacteria are inoculated into a commercially system which have to be frozen according to the manufacturer's instructions. Then, the beads may be removed and placed onto an appropriate nutrient media. Repeated freeze/thaw cycles can compromise the genetic integrity of the organisms [53]. Microorganisms may be cryopreserved [53,57,58], by vitrification and encapsulation [53,59]. In vitrification system, the vitrification solution surrounds the cells forming an amorphous glass; this prevents cryoinjuries. Samples may be rapidly cooled in liquid nitrogen without a system to control cooling rate. The vitrification technique may be used for fungi preservation. In encapsulation process, the cells are embedded in calcium alginate beads prior to cryopreservation. The encapsulation usage has two main benefits: firstly, the water content of cells can be reduced decreasing the risk of ice damages or concentration effects during the cooling stage of the procedure and secondly, it allows cells to be easily handled and manipulated by providing a suiTable suspending matrix. Then, specimens are rapidly cooled without controlled rate cooling [53]. Encapsulation and vitrification are used to preserve recalcitrant microorganisms. Pathogenic or mutualistic microorganisms are preserved with growth substrate or host. For example, hemp seeds have been used to support members of the Chromista when they are cryopreserved. This approach has been used for the microcyclic rust fungus *Puccinia spegazzini* where the teliospores were preserved on petiole tissue [53,60]. Similarly, seeds of the common spotted orchid (*Dactylorhiza fuchsii*) and green-winged orchid (*Anacamptis morio*) were encapsulated in alginate beads with hyphae of the basidiomycete fungus *Ceratobasidium cornigerum* with no adverse effects after cryopreservation [53,61].

Discussion

In the actual research context, scientists need an infrastructure to collect several different samples in order to answer to designed study questions. Sometime, biobank's collections are used for answering to questions raised after study conclusion or to test new technologies. For these reasons, biobanks have to ensure quality of stored specimens.

Actually, millions of samples are collected in European biobanks; unfortunately, these collections are subjected to fragmentation, insecurity of funding and incompleteness. In order to obviate fragmentation, several efforts were needed to create some European networks that joined biobanks across the Europe.

Each sample is managed by local biobank, but it is inserted in virtual catalogue. The creation of virtual catalogues facilitate sharing and accessing to sample's information. The importance of sharing is marked by RDs biobank network. In RDs field, there are few patients and a very little number of samples for each RD. Sharing materials and data is essential for identifying disease-causing genes, studying pathological mechanisms and developing treatment.

The networks are built on pre-existing biobanks; in this context, to harmonize the collections and data annotations is essential. In order to ensure harmonization, for join new biobanks in the network, the biobanks have to adhere to minimum inclusion criteria.

Building European-wide networks ensure:

- Unified catalogue of samples and related date;
- Reduced ambiguity of result interpretation;
- An open-source database;
- European-wide data to guarantee better statistical power;
- Prospective collections;

- Compliance with ethical and legal requirements.

Finally, the collaboration with patient's associations helps the networks to understand how they should function based on patients' life experience, to improve patients' quality life and to respect patients' rights.

The networks should be organized to provide flexibility for facilitating its growth. Membership is non-exclusive but favors members that can link network to other national, European or global initiatives.

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