Report on training in University Certificate in Principles of Biobanking conducted by the University of Luxembourg and the Integrated Biobank of Luxembourg (IBBL)

Introduction

The integrated biorepository of H3Africa Uganda (IBRH3AU) aims to develop state of the art facilities to provide biobanking services to the H3Africa consortium and other researchers. To achieve this IBRH3AU set out to train personnel in biobanking science and management and among other trainings is the University certificate in principles of biobanking offered by the University of Luxembourg and Integrated biobank of Luxembourg.

Course objectives and learning outcomes

At the end of the training, participants should;

- Understand in depth and produce an oral synthesis of the common principles of practical biobanking.
- Put different types of biobanks in perspective and draw conclusions about the theoretical underpinnings that were operational.
- Apply the scientific basis of biobanking/biospecimen research in Standard Operating Procedure (SOP) development and implementation and in research exploitation of samples.
- Question the logistical, practical and technical steps of biobanking, and evaluate their coherence and adequation.
- Compare different reports on biobank risk management and mitigation.
- Develop Best Practices/Standard Operating Procedures (SOPs).
- Validate biobank protocols, training and technology transfers.
- Analyze adequation to biobank Quality Management Systems (QMS) and the principles of certification, quality assurance and 3rd party ISO accreditation.
- Master the regulatory, legal and ethical aspects of biobanking.
- Produce biobank cost analysis and recovery reports.

Facilities

- University of Luxembourg
- Integrated Biobank of Luxembourg

Teaching Methods

Lecture presentations and practical demonstrations

Trainers

1. **Fay Betsou** –IBBL Chief biospecimen scientist
2. **Olga Kofanova -**Cryopreservation specialist
3. **Bill Mathieson-** Biospecimen Expert
4. **Dominic Allen**- IBBL Chief Operating Officer
5. **Kate-** IBBL Biospecimen Shipping Coordinator
6. **Marcos Pestana-** IBBL Functional Application Manager
7. **Marc Vandelaer-** IBBL Chief Information Officer
8. **Laurent Antunes-** IBBL Pathologist
9. **Sabine LEHMANN**-IBBL Quality Manager
10. **Rudi BALLING**- Director, Luxembourg Centre for Systems Biomedicine (LCSB)

IBRH3AU Trainees

1. Dr. Samuel Kyobe- IBRH3AU Coordinator
2. Musinguzi Henry- IBRH3AU Manager

Course content

Definitions, typology of Biobanks

Biobank/biorepository; A facility/infrastructure that transforms and stores tissues and cells and distributes them (free of charge of for a fee) for scientific use.

Scientific Collections Concept e.g. Natural History museums, Herbaria, zoos, Aquaria, botanic gardens

Breeding concept e.g**.** germplasm repositories, plant breeding centers, IVF centers, and genetic stock

Genebanking concept e.g. biospecimen biobanks, DNA banks, blood banks, Field banks est.

Definitions

Specimen: Part of biological solid/liquid from an individual, taken for analysis of specific characteristics and characterization of the whole.

Sample: Part of a specimen which is analyzed or stored e.g. serum, plasma, CSF

Aliquot: Fraction of a sample (tube, straw)

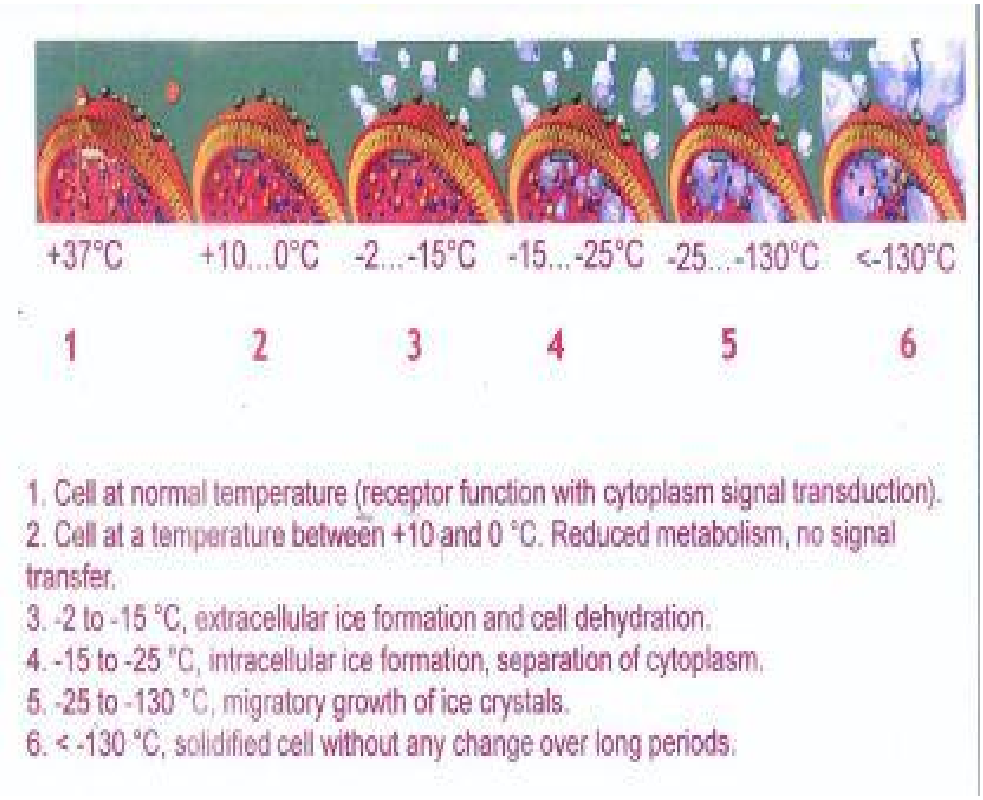
Ice nucleation: Point at which ice crystals are initiated in a sample. Crystallization occurs only when the thermal energy of the system is able to support stable ice nuclei.

Glass transition temperature: Temperature at which a glass is formed and a fluid becomes so viscous that it appears solid.

Super cooling: Process of lowering the temperature of a liquid below its freezing point without it becoming a solid

Freezing point depression: Process when a solution can be cooled below the freezing point of the corresponding pure liquid due to addition/presence of the solute.

Principles of Cryopreservation & Cryoinjury



Cryopreservation: The storage of viable cells, tissues, organs, organisms at ultra low temperatures, usually LN2 or its vapor phase to a minimum of -196^{0}C. Below the point of ***homogeneous ice formation at -40C***

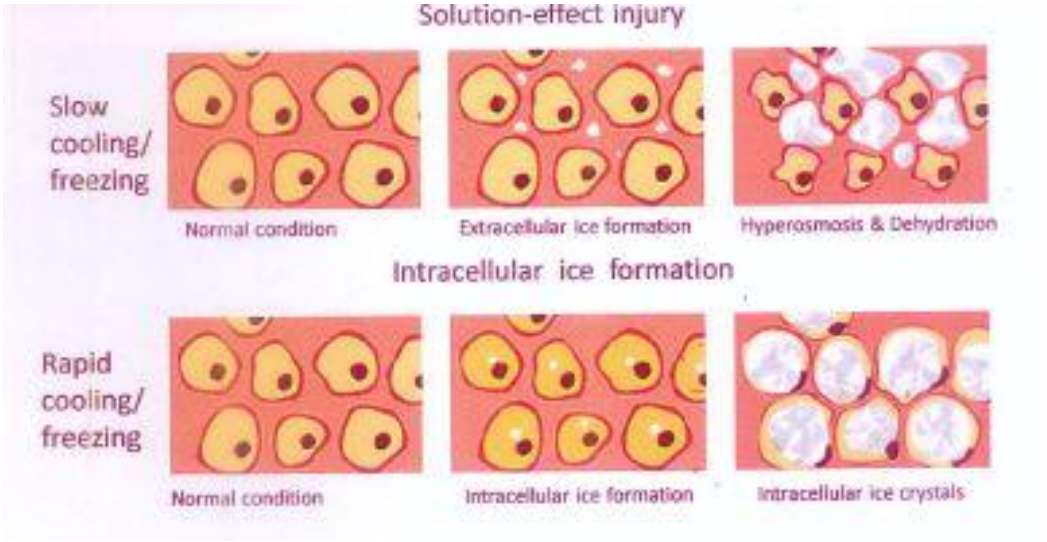
Cryobiology is the study of life at very low temperatures; understanding mechanisms that determine life and death at freezing temperatures and the condition of cells whose ageing process have been arrested by the frozen state.

Ice formed outside the cell causes a vapor pressure deficit between the inside and outside of the cell, this causes water to leaves the cell making it dehydrated reducing the amount of water available for freezing and concentrating solutes which decreases the freezing point inside the cell. When the cell is eventually freezes water left is in such small quantities that ice crystals formed are too small to be lethal.

Importance of cooling rate to cell survival

At slow cooling rates the water molecules have sufficient time to leave the cell once extra cellular ice is formed.

Rapid cooling rates usually result in ice being formed inside and outside the cell at the same time; cells do not have sufficient time for water to leave the cell. The intra cellular ice formed is lethal.



Vitrification

Existence of water in a glassy/vitrified state or when aqueous solutions become highly concentrated to a critical viscosity water molecules can no longer interact with each other to initiate ice crystals on cooling.

Cryoinjury

Mazur's 2-factor hypothesis of cryoinjury

Factor 1; Ice

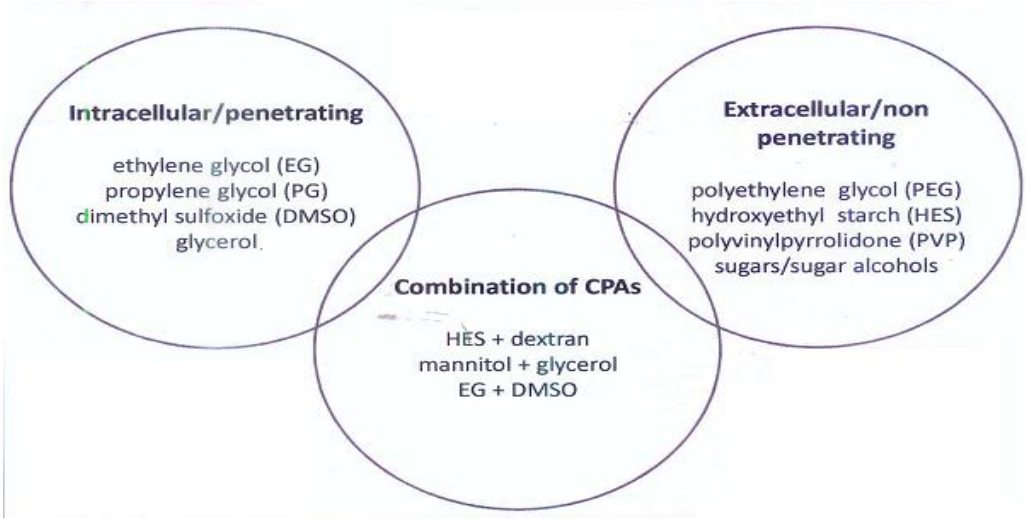
When ice is formed outside the cell, a water vapor deficit is formed across the membrane and water is withdrawn, the cell solutes become concentrated and the freezing point is depressed. When the cell is eventually plunged into the cold (LN2) the water content is so reduced that only small ice crystals form, these don't damage the cell which survives.

Factor 2; Dehydration

As cell solutes become more and more concentrated by freeze dehydration there is a possibility that they become stressed and lethally injured by; **the toxic concentration of the solutes and irrevocably injurious changes in cell volume.**

The two injurious components are determined by the colligative properties of water and its ability to differentially change state in the intra and extra compartments of the cell.

Types/methods of cryoprotectants



Colligative cryoprotection (e.g. DMSO)

The key to colligative cryo-protection is to limit injurious cell volume changes and solute concentration by replacing water with another solvent that; acts as a diluents to prevent toxic concentration of solutes and prevents lethal reduction in cell volume caused by excessive dehydration.

Osmotic cryoprotection (Sucrose, Sorbitol, Mannitol, polyethylene glycols)

Liquid water converts to ice on freezing, osmotic agents assist cryoprotection by reducing the amount of water available to form ice inside the cell. Osmotic cryoprotectants are either non penetrating or limited penetrating.

Vitrification

cryoprotection: objective is to inhibit ice formation completely by increasing cell viscosity to a critical point such that the ability of water to nucleate ice crystals is prevented.

Cooling rate	**Physical response**	**Cryoinjury**
Slow	• Extra cellular ice formation • Water loss/volume reduction	• Cell packing, mechanical damage • Membrane permeability • Ion leakage • Influx of extracellular solute
Rapid	• Dehydration/solute concentration • Supercooling	• Solute toxicity (biomechanical damage) • Intra cellular ice formation (mechanical damage)

Cryopreservation of proteins

Cold denaturation of proteins

Cooling leads to dissociation of various supramolecular structures**, which** reassociate upon incubation at physiological temperatures, low temperature perturbs pka of ionisable groups, increases dielectric content, surface tension and viscosity of the solvent.

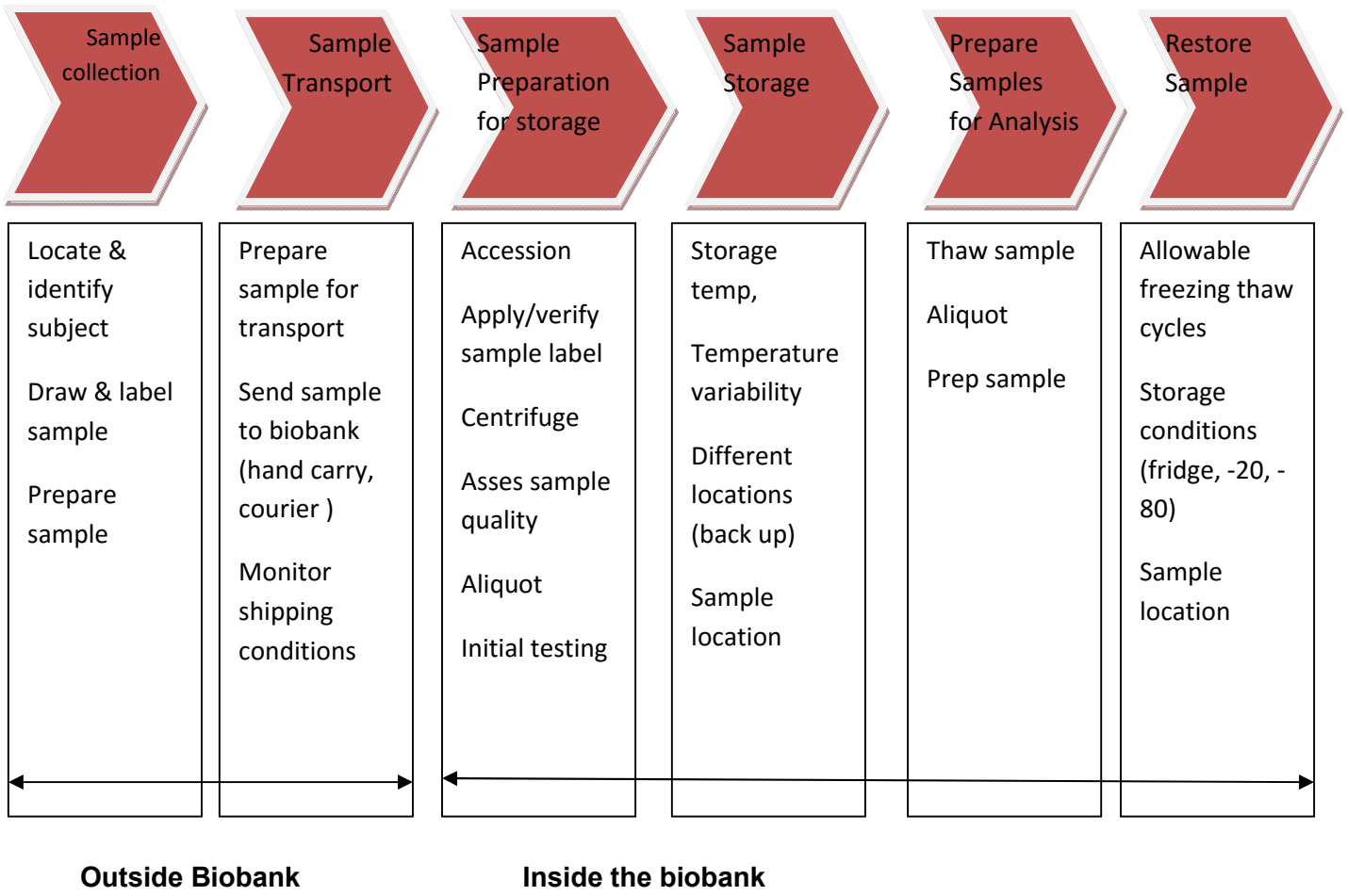
Freeze thawing proteins in presence of cryo-protectants

Loss of activity at high and slow freezing rates

Shear forces exerted on the surface of the protein molecules by ice crystals, slow freezing may result in water crystallization and protein aggregation and weakening of the hydrophobic interactions.

Mechanisms of protein cryoprotection; changing the freezing characteristics of water/modify the nature of ice crystals e.g. PEG makes water more viscous and cryoprotectants which have surface active properties may concentrate at the ice-water interface, preventing surface denaturation of the protein e.g. BSA or Tween80.

Biospecimen; Preanalytical phase



Quality Assurance/Quality Control of Biospecimen

Application	Sample type	QC analyte	Scope/Interpretation	Reference
Proteomics	Plasma	Protein S	Storage duration	Nilson TK et al. Thromb Res 2005,116:249-254, Wel et al Transf Med and hematol 2001, 28:189-194, F Btesou
Proteins	Serum	CD40L	Exposure to RT	F.Betsou 2008
Posttranslational		MMP-7	freeze thaw	F.Betsou, Cilin Chem Lab Med 2007
Metabolomics	RBC	MMP-9	Duration of storage at -80	Rouy D et al Anal Biochem 2005,338:294-298
Enzymes	CSF	Ab42	Freeze thaw or RT exposure	Schoonenboom NSM et al Clin Chem 2005,51:189-195
Vitamins		Cystacin C truncated	storage at -20	Carrete O et al Proteomics 2005,5:3060-65
Oligoelements	Tissues	Vimentin	Antigenicitydegradation	Florrel SR et al Mod Pathol 2001,14:116-128
Lipids		Cyclin-D1	idem	Surjit M, Lal SK Cell Cycle 2007,6:580-588
		Phospho-p27	idem	idem
		2-nitroimidazole	Tissue hypoxia	Raleigh JA et al. Int J Radiat Oncol Biol Phys 1992;22:403-405
		PH	idem	Vonsattel JPG et al. Acta Neuropathol 2008;115:509-32

DNA/RNA	A260/280
	A260/230
	gel electrophoresis
Proteins	Western Blotting
	Mass spectrometry
Cells	Viability
	Sterility

Method validation

Validation: a process by which a procedure is checked against a specific criterion, confirming it meets the intended needs of the user.

Processing method validation where the end result is a sample e.g. DNA, PBMC

(Stability, Robustness)

Reproducibility: comparing results of a protocol replicated several times and robustness
Validation report

Analytical method validation where the end result is a result

Qualitative method

Sensitivity, specificity, contamination between samples, stability, robustness, correlation with reference material or method

Quantitative method

Specificity/interferences, precision, accuracy, sensitivity (Limit of detection, Limit of quantification), linearity, contamination, stability, robustness, matrix effect, reference method or method in use

Cost Analysis & Recovery

Decide and allocate costs for;

Labor

Calculate operator costs for running the tests/process depending on institutional or national guidelines,

Equipment cost calculation model

Equipment	Purchase price	Amort (yr)	Amort/yr	M'ce/yr	Total/yr	hr/d	d/wk	wk/yr	hr/yr	Total Cost/hr
Angilent	4,356	4	1089	54.45	1143.45	8	5	50	2000	0.571725
Centrifuge	5808	4	1452	72.6	1524.6	8	5	50	2000	0.7623
Real Time PCR	46591	4	11647.75	586.89	12234.64	8	5	50	2000	6.11732
Freezer -80	30,000	4	7500	200	7700	8	5	50	2000	3.85
Thermal cycler	7000	4	1750	150	1900	8	5	50	2000	0.95
Qiacube	20000	4	5000	250	5250	8	5	50	2000	2.625

Consumables

Calculate the cost of consumables needed for the process e.g. if one DNA extraction kit of 1000 USD runs 100 samples the cost for one sample would be 1000/100=10 USD

Direct costs e.g. power, water etc.

Decide on overheads percentage to cater for administration costs

Total Cost = Personnel cost + Equipment cost + Consumables cost + Over Heads

Important questions answered

Does the expiry of primary tubes extend when specimens are added e.g. blood samples in RNA/DNA Paxgene?

Stability studies by BD have demonstrated these DNA/RNA samples can last up to eight years after the expiry date indicated on the tube.

Biospecimen Research

The IBBL experience demonstrated the strategy of increasing visibility through publication of biospecimen research data in biobanking journals e.g. Biobanking and Biopreservation journal. This approach will be adopted by the IBRH3AU to enhance biospecimen research, increase visibility.