OPTIMIZATION OF TISSUE SAMPLES FOR THE DEVELOPMENT AND VALIDATION OF DISEASE BIOMARKERS: THE OPTIMARK PROJECT

Villena C[1,2], Artiga MJ[2,3], Bahamonde O[2,4], Belar O[4,5], Bermudo R[2,6], Castro E[4,5], De la Puente R[2,7], Escámez T[2,8], Fraga M[2,9], Jauregui L[2,10], Novoa I[2,11], Peiró-Chova L[2,4], Piñero E[1,2], Rejón JD[2,7], Ruiz M[2,12], Vieiro P[2,9], Zazo S[2,13], Villar V[2,10] y Rábano A[2,14]

[1] *Pulmonary Biobank Consortium, CIBER of Respiratory Diseases (CIBERES) - ISCIII (Madrid), Instituto de Investigación Sanitaria de Palma (IdISPa) - Hospital Universitario Son Espases, Mallorca.*
[2] *Spanish Biobank Platform, ISCIII.*
[3] *CNIO Biobank, Spanish National Cancer Research Center (CNIO), Madrid.*
[4] *INCLIVA Biobank, Valencia.*
[5] *Basque Biobank; The Basque Foundation for Health Innovation and Research (BIOEF), Bilbao.*
[6] *HCB-IIDIBAPS Biobank, Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona.*
[7] *Andalusian Public Health System Biobank, Granada.*
[8] *IMIB Arrixaca Biobank, Murcia.*
[9] *Biobank of Research Institute of Santiago de Compostela, Santiago de Compostela.*
[10] *University of Navarra's Biobank IdiSNA, Pamplona.*
[11] *Biobanco Hospital Universitario Valld'Hebron, Vall d´Hebrón Institut de Recerca (VHIR), Barcelona.*
[12] *IRBLleida Biobank, Instituto de Investigaciones Biomédica de Lleida-Fundación Dr. Pifarre, Lérida.*
[13] *Fundación Jiménez Díaz Biobank, Madrid*
[14] *Departamento Neuropatología, Banco de Tejidos Cien, Fundación Centro Investigación Enfermedades Neurológicas (CIEN), Madrid.*

INTRODUCTION

The development of new disease biomarkers is essential for the progress of personalized medicine. This clinical approach, allows a great number of patients to access more efficient and safer therapeutic protocols, based on molecular findings in tissue samples obtained from patients for diagnostic or therapeutic purposes.

Biobanks, as repositories of human biological samples, have the responsibility to provide researchers with samples of known quality and an estimated potential for the analysis of DNA, RNA, proteins and antigenicity. A key element in this context is the knowledge, control and registration of all pre-analytical factors related to the final analytical performance of samples.

This issue is particularly complex when dealing with samples from a wide range of tissues, obtained through various procedures (biopsy, resection or post-mortem) and potentially tested with different analytical procedures.

OPTIMARK PROJECT

The purpose of the project, that is currently being carried out by 17 centres of the Spanish Network of Biobanks, is to select and validate the essential preanalytical variables relevant in analytical testing and high predictive value for the applicability of research samples of new tissue biomarkers.

The project will allow a retrospective analysis of the potential of tissue samples stored in the Network, and the effectiveness of the current codification systems that include pre-analytical variables. Additionally, the project aims at developing optimized protocols for obtaining samples of various tissue origins, controlling key pre-analytical factors (**Fig. 1**), and the evaluation (algorithms) of analytes (generic and specific) related to biomarkers (**Fig. 2**).

In the first phase, 374 patients between 30 and 65 years (in the case of brain samples up to 75 years without vascular pathology) have been selected to test their tissue samples in order to evaluate long term storage based on RNA and antigenicity quality. A representative number of frozen and formalin-fixed paraffin-embedded tissue samples stored less than 1 year, 1-5 years, 5-10 years and more than 20 years have been selected from colon (n=104), brain (57), lung (76), breast (85), stomach (17) and endometrium (35).

Correlations will help to analyse the impact of other key preanalytical factors.

EXPECTED IMPACT OF PRE-ANALYTICAL FACTORS ON TISSUE QUALITY

		RNA/DNA									Antigenicity								
	ITEM	Anatomical or collection site	Body temperature before tissue collection	Type of collection	Warm ischemia time	Cold ischemia time	Fixation / stabilization type	Fixation/Stabilization time	Long-term storage	Storage duration (between acquisition and distribution)	Anatomical or collection site	Body temperature before tissue collection	Type of collection	Warm ischemia time	Cold ischemia time	Fixation / stabilization type	Fixation/Stabilization time	Long-term storage	Storage duration (between acquisition and distribution)
Anatomical site	colon, lung, liver, mama																		
	fatty, fibrous, pancreatic, neuronal																		
Body temperature	4ºC (post-mortem)																		
	Room temperature 18-28ºC (post-mortem)																		
	37ºC (alive)																		
	Unknown																		
Type of collection	Autopsy < 6 h post-mortem			A06									A06						
	Autopsy 6-12 h post-mortem			A12									A12						
	Autopsy 12-24 h post-mortem			A24									A24						
	Autopsy 24-48 h post-mortem			A48									A48						
	Autopsy 48-72 h post-mortem			A72									A72						
	Biopsy in culture media			BCM									BCM						
	Biopsy			BPS									BPS						
	Biopsy in normal saline or phosphate buffered saline			BSL									BSL						
	Biopsy in tissue low temperature transport media			BTM									BTM						
	Punction			PUN									PUN						
	Surgical excision in culture media			SCM									SCM						
	Surgical excision			SRG									SRG						
	Surgical excision in normal saline or phosphate buffered saline			SSL									SSL						
	Surgical excision in tissue low temperature transport media			STM									STM						
	Surgical excision in vacuum container			VAC									VAC						
	Swab			SWB									SWB						
Warm Ischemia Time	< 2 min				A									A					
	2 to 10 min				B									B					
	10 to 20 min				C									C					
	20 to 30 min				D									D					
	30 to 60 min				E									E					
	>60 min				F									F					
	Unknown				X									X					
	Not applicable (e.g. biopsy, postmorten)				N									N					
Cold Ischemia Time	< 2 min					A									A				
	2 to 10 min					B									B				
	10 to 20 min					C									C				
	20 to 30 min					D									D				
	30 to 60 min					E									E				
	>60 min					F									F				
	Unknown					X									X				
	Not applicable					N									N				
Fixation / stabilization type	Non-aldehyde with acetic acid						ACA									ACA			
	Aldehyde-based						ALD									ALD			
	Allprotect ® tissue reagent						ALL									ALL			
	Alcohol-based						ETH									ETH			
	Non-buffered formalin						FOR									FOR			
	Heat stabilization						HST									HST			
	Snap freezing						SNP									SNP			
	Non-aldehyde based without acetic acid						NAA									NAA			
	Neutral buffered formalin						NBF									NBF			
	Optimum cutting temperature medium						OCT									OCT			
	PAXgene® tissue						PXT									PXT			
	RNA Later®						RNL									RNL			
Fixation/Stabilization time	<15 min							A (ALL)	A (NBF)	A (PTX)	A (RNL)							A (NBF)	A (PTX)
	15 min to 1 h							B (ALL)	B (NBF)	B (PTX)	B (RNL)							B (NBF)	B (PTX)
	1 to 4 h							C (ALL)	C (NBF)	C (PTX)	C (RNL)							C (NBF)	C (PTX)
	4 to 8 h							D (ALL)	D (NBF)	D (PTX)	D (RNL)							D (NBF)	D (PTX)
	8 to 24 h							E (ALL)	**E (NBF)**	E (PTX)	E (RNL)							E (NBF)	E (PTX)
	24 to 48 h							F (ALL)	F (NBF)	F (PTX)	F (RNL)							F (NBF)	F (PTX)
	48 to 72 h							G (ALL)	G (NBF)	G (PTX)	G (RNL)							G (NBF)	G (PTX)
	Not applicable (OCT/Snap Frozen)							N	N	N	N							N	N
	Unknown							X	X	X	X							X	X
	Other							Z	Z	Z	Z							Z (NBF; brain	Z
Long-term storage	PP tube 0.5- to 2-mL** (-85) to (-60) °C								A										A
	PP tube 0.5- to 2-mL (-35) to (-18) °C								B										B
	PP tube 0.5- to 2-mL <-135 ° C								V										V
	Cryotube 1- to 2-mL Liquid nitrogen***								C										C
	Cryotube 1- to 2-mL (-85) to (-60) °C								D										D
	Cryotube 1- to 2-mL Programmable freezing to <-135 °C								E										E
	PP tube ≥ 5 mL (-85) to (-60) °C								J										J
	PP tube ≥ 5 mL (-35) to (-18) °C								K										K
	Cryotube 1- to 2-mL LN*** after temporary (-85) to (-60) °C								N										N
	Paraffin block RT or 2 to 10°C								P										P
	Bag LN***								G										G
	PP tube 40 to 500µL (-85) to (-60) °C								S										S
	PP tube 40 to 500µL (-35) to (-18) °C								T										T
	PP tube 40- to 500-µL <-135 ° C								W										W
	Original primary container (-35)-(-18) °C or (-85)-(-60) °C								Y										Y
	Unknown								X										X
	Neutral buffered formalin (ex. Brain)								Z										Z
	Other *(ex.Cryomold)*								Z										Z
Storage duration	< 5 years																		
	5 - 10 years																		(NBF)
	10 - 20 years																		(NBF)
	> 20 years																		(NBF)

Figure 1. Expected impact by colours of selected SPREC v.2.0 (gray cell) and BRISQ (blue cell) preanalytical factors on tissue quality, over the nucleic acids and antigenicity. Green: optimal quality or not effect; yellow: moderate quality or unknown effect; red: sub optimal quality.

PROPOSALS AND CONCLUSIONS

1. The SPREC coding system and a subset of BRISQ variables have been used to estimate the expected quality for each analyte group (**Fig. 1**).

2. The Research Group has addressed strategies independently, and a decision tree has been developed to assess the quality of each analyte group (**Fig. 2**).

3. Selected retrospective samples are being analysed in order to validate the proposed procedure, to evaluate the effect of several preanalytical factors, as well as the cost-efectiveness in tissue biomarkers research.

4. A prospective tissue sampling phase shall be required to validate the gaps on available information.

ALGORITHMS PROPOSED TO TEST QUALITY

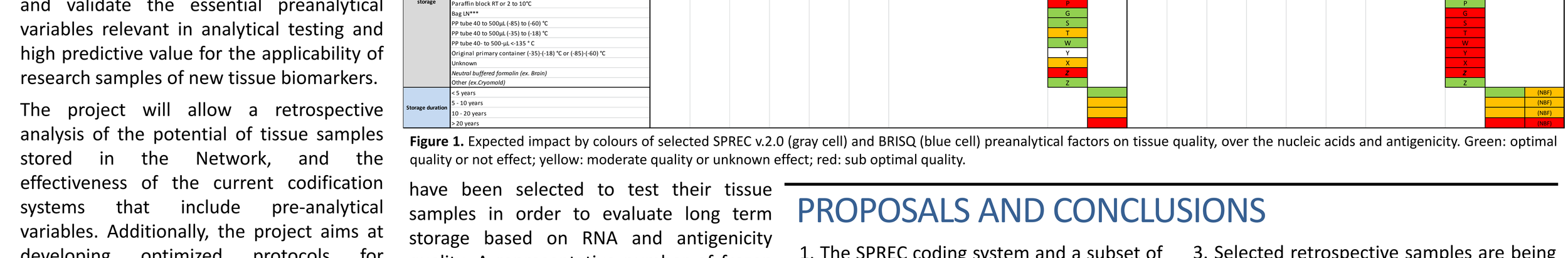

Figure 2. Procedures to evaluate tissue quality, in order to classify the suitability of the sample for the expected application. Green: optimal quality; yellow: moderate quality; red: sub optimal quality.

INSTITUTIONS