



## Scientific -Technical Service of Immunohistochemistry

**RULES AND INSTRUCTIONS**

## Scientific -Technical Service of Immunohistochemistry

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## Scientific -Technical Service of Immunohistochemistry

### 1. Objectives

1. To be a useful tool for research.
2. To support researchers to achieve their goals with optimal quality.
3. To design new protocols.
4. To use Tissue Micro Arrays (TMAs) technology.
5. Complete analysis of results using ACIS® III (Automated Cellular Imaging System).
6. To perform external quality controls.
7. To perform basic training sessions for service users, as well as training courses in the framework of Universitat de Lleida.
8. To establish an agile working dynamics to cover research needs at any time.

### 2. Instalation and equipment

Sample processing is performed according established laboratory protocols. There are three apparatus (Immunostainings) that belong, one of them to IRBLleida and the two others to Hospital Universitari Arnau de Vilanova. Each apparatus, (Autostainer Link 48 y OMNIS, DAKO), always connected to a computer to monitor its functionality, allows the processing of up to 48 slides. The laboratory is equipped with an apparatus for image analysis (ACIS® III Instrument, DAKO) with ACIS® III Software, an instrument to manually perform Tissue Micro Arrays (TMA) (Beecher Instruments TMA apparatus), and to automatically perform TMAs (TMA Grand Master (3D HISTECH), additionally it disposes of an apparatus (PTLink, DAKO) for the pre treatment of samples, a microtome, a biological safety cabinet, a microscope, three combi-fridge freezers, a kit of pipettes, etc.

### 3. Types of samples

The Immunohistochemistry Scientific and Technical Service allows the processing of:

- Tissues (human, animals) (paraffin embedded)
- Tissues (frozen)
- Citology specimens
- *In vitro* cell cultures

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### 4. Obtaining and registering samples

#### - Obtaining

In relation to human tissue samples, their access to the Service is carried out through the Biobank of IRBLLeida.

In relation to animal samples and in vitro cell cultures, the Principal Investigator provides them to the assigned technician.

#### - Registering

The Service records all tasks. The devices of the Service have their own specific software for storing data. Only Immunohistochemistry Service personnel have access to this data.

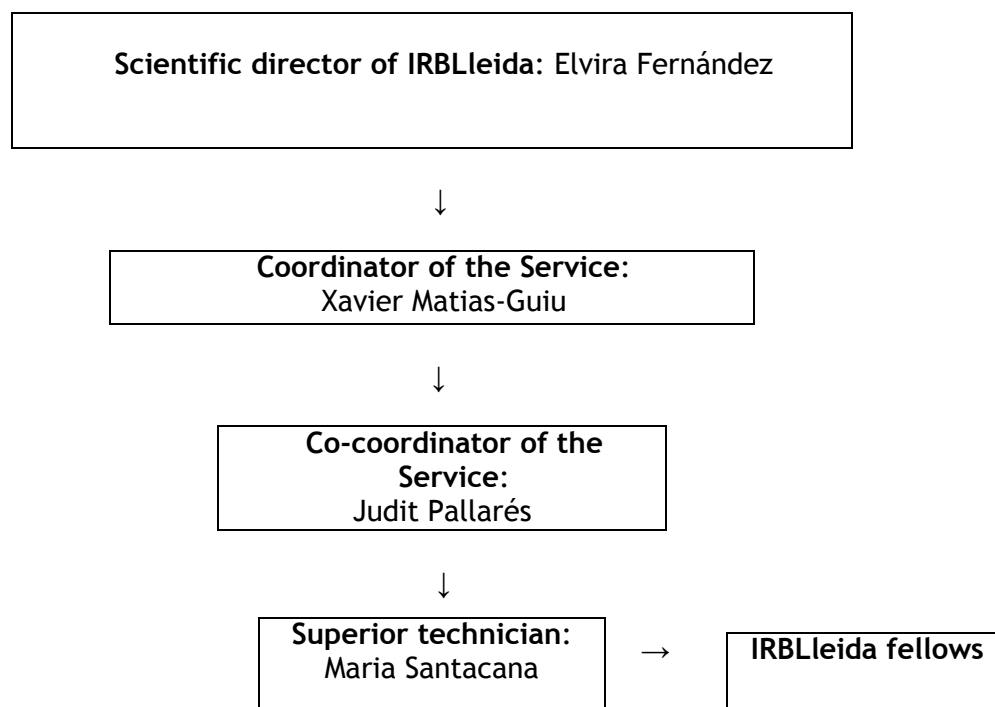
### 5. Organization

The Immunohistochemistry Service of IRBLLeida is an organizational structure that depends directly on the scientific director of IRBLLeida.

#### a. Staff organization chart

The daily work team of the Scientific-Technical Service of Immunohistochemistry is composed of a superior technician with full dedication, IRBLLeida fellows with partial dedication and the coordinators of the Service with partial dedication. The technician depends directly on the coordinator and the co-coordinator of the Service.

## Scientific -Technical Service of Immunohistochemistry



The functions of the coordinator and co-coordinator of the Immunohistochemistry Service are as follows:

- To promote the activity of the Service between researchers, internal and external research groups, companies, etc.
- To lead and promote the own investigation of the Service.
- To ensure that the operating rules of the Service are accomplished.
- To lead and verify the budgets assigned to the service activity.
- Convening and attending internal meetings related to the Service.
- To lead and advise the assigned superior technician.

The functions of the assigned superior technician are as follows:

- To ensure that the operating rules of the Service are accomplished.
- To make the budgets assigned to the service activity.
- Attend to the users whenever they need it.
- To advise and train users.
- To apply standard work procedures.
- To attend internal meetings of the Service.
- To give results as fast as possible after setting the criteria of priorities.
- Perform the internal investigation of the Service.
- Be responsible for the good daily operation of the Service.

## **Scientific -Technical Service of Immunohistochemistry**

### **b. Users**

The users of the Immunohistochemistry Service are the assigned superior technician and the IRB fellows (with prior authorization of their IP (principal investigator) and the coordinator of the Immunohistochemical Service). They will have to go through a previous training period.

### **c. Beneficiaries**

The direct beneficiaries of the Immunohistochemistry Service are:

- Internal research groups of IRBLleida
- Research groups from external centers
- Groups / private centers / companies

## **6. Quality controls**

### **a. Internal quality controls**

Internal quality controls are performed routinely within the Service. The tissues that need to be studied are good, most of the time, to validate the technique. In addition, tissue sections or cell samples acting as positive and negative controls are added.

### **b. External quality controls**

External quality controls can be classified into two types: those performed annually by external agencies or those performed by the Service itself for other centers.

For the first group, the results of the techniques are externally evaluated; As a consequence, it allows the improvement of the protocols and the possibility of offering a better service.

For the second group, it is the Service itself that evaluates the immunohistochemical techniques of other organisms. This allows us to act as a Reference Center and is an indicator of the quality of daily work.

## **7. Rules and instructions**

### **a. Ethical aspects**

## **Scientific -Technical Service of Immunohistochemistry**

The organism responsible for verifying the ethical, quality and feasibility of each scientific project is the Committee for Clinical Research (CEIC) and the Ethical Committee for Animal Experimentation (EAC), always in accordance with the active legislation:

- Biomedical research law 14/2007
- Organic Law 15/1999 on the protection of personal data
- Convention of the European Council for the Protection of Human Rights and Human Dignity concerning the applications of biology and medicine, (Spain ,1 January 2000).
- Additional Protocol to the Convention on Human Rights and Biomedicine, in relation to biomedical research (2005)
- Recommendation of the Council of Europe on research on biological material of human origin of 15 March 2006.
- Guide to good practice in health science research. Institut Català de la Salut, 2015.
- Biosafety in Microbiological and Biomedical Laboratories; US Department of Health and Human Services, 4th edition.

All clinical information will be anonymous and confidential. The Service will ensure the integrity and confidentiality of all data files provided.

### **b. Rights and duties of users of the Immunohistochemistry Service**

Before starting the experiments the user will have to follow the following steps:

- To perform training prior to conducting the experiments; This will be given by the assigned Technician.
- To inform the assigned Technician of what is wanted to be done in order to be able to organize the tasks in the best possible way.
- To keep the Service in perfect condition. After working, leave the Service to the point so that new users can work. Take responsibility for the site and the working tools.
- Consult any incidence and any doubts with the assigned Technician.

### **c. Rights and duties of the staff of the Immunohistochemistry Service**

- The staff of the Immunohistochemistry Service is responsible for the organization and daily operation of the Service.

## Scientific -Technical Service of Immunohistochemistry

- Provide adequate training to new users of the Service.
- Advice any incidences and any doubts with the coordinator and co-coordinator of the Service.

### 8. Services and benefits

The applications currently available to the Immunohistochemistry Service are:

- Immunohistochemical protocol for the detection of proteins in human and mouse tissues by concentrated or pre-diluted antibodies produced in goat, mouse or rabbit, etc. (see antibodies available in ANEX 1: Listing 1, Listing 2).
- Construction of Tissue Micro Arrays (diameters cylinders from 0.6mm to 2mm).
- Immunohistochemical double staining protocol. Simultaneous 2-protein detection in the same section of tissue.
- Immunofluorescence technique.
- Special stains (Histochemistry).
- Histological stains (Hematoxilin-Eosin).
- Analysis of images of complete sections or Tissue Micro Arrays using the ACIS® III Instrument.
- *In situ* hybridization using FISH (Fluorescent In Situ Hybridization) or CISH (Chromogenic In Situ Hybridization).
- Quality controls.

To develop other techniques that are wished to perform is not discarded.

### 9. Service requests

- a. The Principal Investigator or applicant will contact the service coordinator or the assigned superior technician.
- b. A budget will be provided to the applicant in accordance with the required services (In the IRBLLeida web site budgets can be consulted).
- c. The service request will then be formalized. To the IRBLLeida website you can find the document "Sol·licitud de servei".

### 10. Budget

In the IRBLLeida web site budgets can be consulted



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### 11. ANNEX 1

LIST 1 PRIMARY ANTIBODIES	
ALDH1 anti mouse	KSR1
ALDH1	LPCAT1
ANNEXIN II	LYVE1 (rabbit anti-mouse)
ARID1A	MMP1
BAD	Mtor
BAP1	MUSASHI
BCATENIN	N-CADHERIN
BNIP3	NFKB P65
CANNABINOID RECEPT I	NFKB P65
CANNABINOID RECEPT II	NFKB phospho P65
CLEAVED CASPASE 3	Nestin
CASPASE-3	NICASTRIN
ACTIVE CASPASE-3	activated NOTCH1
CD11B	NRF2
CD105	Oct-4
CD123	PD1
CD133	PDGFR ALPHA
CD163	PD-L1
CD20 (rabbit anti mouse)	Phospho-Histone H3
CD3E (goat anti-mouse)	p105/p50
CD31 (rat anti mouse)	p110/p52
CD31(rabbit anti-mouse)	Pakt
CD33	PERK
CD44	PMTOR (SER2448)
CD59	Ps6k1
CD8	P4ebp1
CD8	P14ARF
CD9	p21
CD11B anti mouse, human	p27
CD11c (hamster anti-mouse)	P65
CLDN3	PP65
CLDN4	PNP
c-MYC	PRESENILIN 2
CHK1 (phospho S345)	Proteasome b4
CYP2R1	Pvegfr3
CYP24A1	RASSF1A
CYP27A1	RB
Cyclin E	SOX2
DCR1	SPOP
EGFR(E746-A750del)	STK15/6-AIK-AURKA
EGFR(L858R Mutant)	TCR g/d (AB ONLINE)
FGFR1	TCR g/d (NV)
FGFR2	TELOMERASE
FGFR4	TLR4
FGFR4	UCKL1
FLIP S/L	VDR
FOXP3	XANTINE OXIDASE
GUANINE DEAMINASE	
H2AX (phospho S139)	
HIF1A	
HIF1A	
HIF1A	
HSP70	
HSP90	
ki 67( rat anti-mouse)	
Ki67 (rabbit anti-mouse)	

## Scientific -Technical Service of Immunohistochemistry

LIST 2 PRIMARY ANTIBODIES		
(Muscle) Actin	Cytoqueratin 17	Myogenin
(Smooth Muscle) Actin	Cytoqueratin 19	Napsin A
Alpha-inhibin	Cytokeratin 20	NSE (Eolasa)
Alpha-1-Fetoprotein	Cyclin D1	Neurofilament Protein
ALK protein	C-MYC	OCT 3/4
ALK	D2-40	P40
Amyloid A	Desmin	PAX2
Androgen Receptor	Dpc4	PAX5
ARID1A	DOG-1	PAX8
B- Catenin	EGFR	PD1
Ber-Ep4	E-cadherin	PLAP
Bcl-2	EMA	PMS2
Bcl-6	Epithelial-related antigen	Progesterona Receptor
BRG-1	ERG	PSA
Calcitonin	Estrogen Receptor	PTEN
Caldesmon	Gastrin	P16
Calretinin	GATA3	p53
CD1a	GCDFP15	P57
CD2	GFAP	P63
CD 3	GLIPICAN-3	P504S (Racemase)
CD 4	GLICOPHORINA A	Myosin
CD 5	GLUT1	SALL4
CD 7	Granzime B	SDHA
CD 8	Helicobacter Pylori	SDHB
CD 10	Hepatocyte	Somatostatin
CD 15	Herpes Virus I	SOX10
CD20cy	Herpes Virus 8	Synaptophysin
CD 21	HBsAg	S-100
CD 30	IDH1	TDT
CD 31	HER2	TIA-1
CD 34	HIK1083	Thyroid Cancer Marker
CD 43	HMGA2	Thyroglobulin
CD 45 RA	HNF1 $\beta$	TLE
CD 45 RO	IgA	TTF-1
CD 45 (leucocitari comú)	Ig D	Villin
CD56	Ig G	Vimentin
CD 61	IgG4	WT-1
CD 68	IMP2	
CD 79 alfa	IMP3	
CD 99	INI-1	
CD 117 (c-Kit)	Insulin	
CD 138	Kappa	
CDK4	Ki-67	
CDX2	Lambda	
CEA	LMP	
Chorionic Gonadotropin (Bhcg)	Mammaglobin	
Chromogranin A	MDM2	
CMV	Melan-A	
Colagen IV	Melanosome (HMB-45)	
Colagen XI	MLH-1	
Cytokeratin AE1/AE3	MSH-2	
Cytokeratin HMW (prostàtica)	MSH-6	
Cytokeratin 7	MUC1	
CK5/6	MUC2	
Cytoqueratin 14	MUM1	
Cytoqueratin 15	Myeloperoxidase	