

# HISTOPATHOLOGY CORE UNIT

Vacant  
Core Unit Head

Staff Scientist  
Eduardo José Caleiras (since March)



## OVERVIEW

Pathology is devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs that underlie disease. By using molecular, immunological and morphological techniques, pathology aims to serve as the bridge between basic science and clinical medicine.

The Histopathology Unit offers assistance and expertise through a full range of services covering from paraffin embedding and tissue sections to histochemical stains, research and diagnostic immunohistochemistry (IHC) testing, antibody validation, and *in situ* hybridisation (ISH), as well as the construction of tissue microarrays. Furthermore, the Unit offers other value added services assisted by a team of highly specialised technicians, such as laser-capture microdissection; slide digitalisation for bright-field, polarisation light and

**“The implementation of multiplexed immunohistochemistry staining to enable the detection of several markers on the same tissue section is an example of the compromise of the Unit with innovation to facilitate the progress of research projects at CNIO.”**

fluorescence; image analysis; and quantification. The Unit collaborates with researchers at any stage of their scientific career in the histological characterisation of phenotypically relevant animal models of disease, thus providing them with the pathology expertise required for the success of their projects.

Technicians  
Nuria Cabrera, María Gómez, Patricia González, Gabino Hernández, Vanessa Pizarro (PEJ, CAM) \*, Irene Roda, Zaira Vega

\*Plan de Empleo Joven de la Comunidad de Madrid (Youth Employment Plan, Community of Madrid)

## RESEARCH HIGHLIGHTS

In line with the activity carried out during the last years, the Unit has maintained the portfolio of services demanded by its users in accordance with the needs of their projects. Thus, about 34,000 paraffin blocks of tissue samples were generated, and ca. 35,000 techniques performed, including histological and IHC techniques, *in-situ* chromogenic hybridisation, tissue microarrays, slide scanning, etc. Also, during this time we introduced new IHC markers useful for the study of tumour development, as well as new chromogenic substrates for the visualisation of those markers.

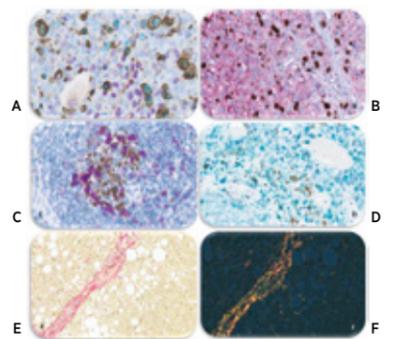
Our main key goals are to guarantee the standardisation and quality of the techniques offered by the Unit, and to focus on the implementation of innovative methodologies. The implementation of new equipment for the routine techniques has been key for the standardisation of the protocols. This has made it possible to introduce new approaches, such as multiplexed IHC staining, which enables the simultaneous visualisation of several markers (up to 4) on the same tissue section. With this new methodology, combined with the slide scanner AxioScan and the ZEN image analysis software available at the Unit, it is now possible to study the distribution and expression levels of different proteins of interest in a single experiment in order to better understand complex pathological processes.

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. In this respect, it is worth mentioning that our haematoxylin-eosin staining technique was commended in the framework of the evaluation, carried out by UK NEQAS, of our histochemical

techniques. On the other hand, NordiQC has evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers and PD-L1; these all obtained very high scores.

Training and outreach activities are also a critical component of the activity of the Unit. This includes our participation in modules of *Formación Profesional* for pathology technicians, mentoring of high school students in short-term stays at the Unit, guided visits to the laboratories for students and other audiences, as well as offering practice sessions on the different technologies mastered by the Unit in Masters and other courses, among other activities. ■

**Figure** Co-expression of different proteins in the same tissue section can be assessed by means of multiplexed IHC staining under brightfield. (A) Triple multiplexed staining of Nestin (brown) + phospho-STAT3 (purple) + p21 (light blue) on fibrosarcoma tissue. (B) Dual staining of Ki-67 (brown) + c-MET (red) on melanoma tissue. (C) Dual staining of BCL-6 (brown) + PD-1 (purple) on lymphoid germinal centre tissue. (D) Visualisation of HMB45 expression in melanoma tissue using a new chromogenic substrate from the Roche Discovery Teal HRP kit (light blue).



**Histochemical techniques are often a quick way to analyse the properties of the tissues. Sirius red staining of fibrotic liver under brightfield (E) and polarised light (F) allows visualising collagen and amyloid deposits.**

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