

Histology and Immunohistochemistry Unit

Summary:

The Unit is a Core Facility for histopathology analysis, providing the most up-to-date technologies within this field for both research and the diagnosis of human tumour samples in order to contribute to better diagnosis and also to facilitate the translation of basic discoveries into clinical applications. We are committed to providing the highest quality in histological, immunohistochemical, chromogenic *in situ* hybridisation (CISH), image digitalisation and quantification analysis, laser microdissection and tissue microarrays production, and supporting both clinical and basic research.


Currently, the Unit has an inventory of over 30 automated histological techniques, 700 primary antibodies and a Tissue Microarray Bank. To maintain gold standard quality, the Unit has ISO 9001 Certification and participates in the External Quality Control Scheme: CECP from SEAP.

Main objectives:

- To provide high quality histopathology services and tissue microarrays production; developing and implementing new technologies and protocols
- To maintain and increase the antibody bank, characterising new and interesting antibodies
- Establish a gold standard procedure for new CISH probes and to optimise *in situ* detection of miRNAs
- To obtain ISO15189 accreditation for clinical laboratories

Lydia Sánchez

Unit Head



Lydia Sánchez born in Toledo, Spain, received her Degree in Biological Sciences from the *Universidad Complutense* in Madrid in 1985. In 1987 she was appointed as Fellow at the Department of Genetics, the *Hospital, Virgen de la Salud*, Toledo, where she began to work with molecular genetic and cytogenetic techniques.

From 1990-1995, she worked on the immunophenotypic characterisation of lymphomas and leukaemias, and minimal residual disease (MDR) in B-ALL. Several clone-specific probes were produced to follow-up and detect minimal residual disease in B-ALL patients in clinical remission.

In 1996, she was responsible for setting up immunohistochemistry procedures and leading the first Spanish Agency of Quality Control in immunohistochemistry (ACCIHQ).

In February 2000 she joined the CNIO as Head of Histology and Immunohistochemistry Unit and set up tissue microarray technology as a high-throughput tool in clinical cancer research. From 2001-2008 she has been involved in several educational programmes including training in molecular pathology for MIRs, short courses (basic and advanced) in molecular pathology and workshops on tissue microarray technology.

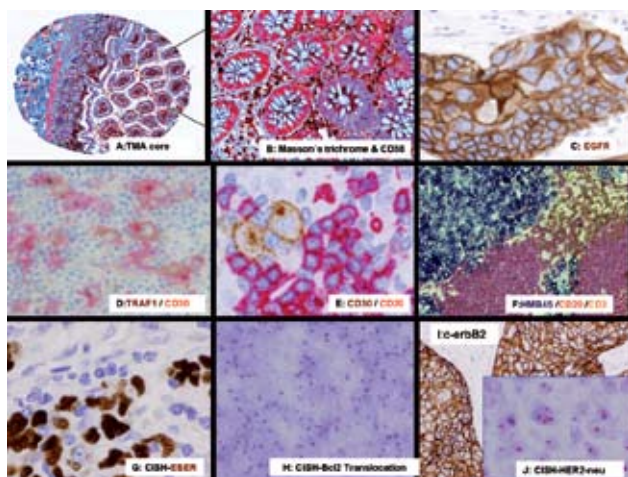
She is Assessor for several Immunohistochemistry Quality Control programmes of the Spanish Agency: *Sociedad Española de Anatomía Patológica* (SEAP), and has contributed to over 50 articles published in renowned international publications.



Technicians:
David Álvarez
(until February)
María J. Campillo
Ana Díez
Raquel Pajares
Zaira Vega

Equipment, techniques and technical implementation

The Unit has implemented automated protocols for Chromogenic *in situ* Hybridisation, DuoCISH™, along with double immunostaining in combination with CISH. DuoCISH™ is optimised for the detection of gene amplifications, deletions and translocations. CISH offers gene status evaluation simultaneously with tissue morphology, in bright-field microscopy and allows archivable and quantitative results.



Figure

A & B: Double staining in a TMA core C: EGFR in Epidermoid carcinoma.
D & E: Double immunostaining in Hodgkin Lymphoma.
F: Triple immunostaining in Malignant Melanoma.
G: Chromogenic *in situ* Hybridisation: EBV EBER
H: Bcl2 gene translocation.
I, C-erbB2 over expression J: Amplified HER2 gene status (CISH) in the same tissue sample.

Our Unit provides a wide range of tools including full slide digitalisation, image analysis, 'virtual microscopy' DOTSLIDE and Laser Microdissection. Furthermore, ARIOL - a high throughput automated image analysis system for quantification in research, clinical, and proteomic applications, has proven an extremely useful tool for quantifying markers in tissue microarrays.

Since Laser Microdissection (LMD) is a powerful resource to sort cell populations, we have also implemented a highly sensitive detection method for EGFR mutations.

The Unit is equipped with:

- Palm Microlaser Microdissector
- Ariol: slide digitalisation and quantification system
- Dot Slide
- 3 DAKO immunostainer & 2 LEICA-Vision Biosystem (BOND)
- Beecher ATA-27 Tissue arrayer

Research activities

Our research surrounds two key activities: antibody characterisation to analyse their utility in diagnosis, prognosis or therapy, using tissue microarray and image analysis quantification technology, and secondly, tissue microarray production for target validation internal quality control and antibody characterisation.

We also collaborate in a Laboratory Training Programme on TMAs histopathology workshops, Master in Molecular Oncology, and an external quality control scheme (SEAP).

Certifications:

- External Quality Control Scheme the *Sociedad Española de Anatomía Patológica* (SEAP)
- ISO 9001. ■

Publications

Montes-Moreno, S., Roncador, G., Maestre, L., Martínez, N., Sanchez-Verde, L., Camacho, F.I., Cannata, J., Martínez-Torrecuadrada, J.L., Shen, Y., Chan, W.C., and Piris, M.A. (2008). Gcet1 (centerin), a highly restricted marker for a subset of germinal center-derived lymphomas. *Blood* 111, 351-358.

Nam-Cha, S.H., Roncador, G., Sanchez-Verde, L., Montes-Moreno, S., Acevedo, A., Domínguez-Franjo, P., Piris, M.A. (2008). PD-1, a follicular T-cell marker useful for recognizing nodular lymphocyte-predominant Hodgkin lymphoma. *Am J Surg Pathol* 32, 1252-1257.

Díaz-Alderete, A., Doval, A., Camacho, F., Verde, L., Sabin, P., Arranz-Sáez, R., Bellas, C., Corbacho, C., Gil, J., Perez-Martín, M., Ruiz-Marcellán, M., Gonzalez, L., Montalbán, C., Piris, M.A., Menarguez, J. (2008). Frequency of BCL2 and BCL6 translocations in follicular lymphoma: Relation with histological and clinical features. *Leukemia Lymphoma* 49, 95-101.