

# Reliable histopathological diagnoses

**Professor Anna Sapino** aims to improve the pre-analytical phases of tissue specimen management, thereby ensuring that correct diagnoses are made. Working in cancer pathology, she describes a novel method to prevent biomarker degradation



## How has your professional background benefited your current research?

Diagnostic standardisation has always been a priority of the pathology lab I lead. From a research standpoint, I consider the translation of experimental results into routine practice essential. With this aim in mind, I have been a principal investigator and collaborator in several scientific projects to validate biomarkers and develop new molecular tests on human tissues. I lead a consolidated research group and collaborate with basic and clinical scientists with different backgrounds, including biochemical and biological sciences, biotechnology and oncology. This multidisciplinary interaction has paved the way for my studies on the pre-analytical phase in surgical pathology, which represents the starting point for an optimal diagnosis and may affect the reliability of research outcomes.

## Could you provide a brief summary of your work?

I am a pathologist and, as such, have to make accurate histopathological diagnoses. However, a pathologist must also be able to

link morphology to the biology and molecular pathways of diseases. In recent decades, the role of pathologists has changed; we are now involved in the clinical management of patients by offering reliable results and the most accurate and innovative tests for patient care. This is particularly true in oncology, where the pathologist plays a crucial role. My specialisation is breast cancer. Depending on the pathological diagnosis, the patient may be offered at least three different treatments: hormone therapy, target therapy with anti-HER2 compounds or chemotherapy. When I am performing a diagnosis, I must keep clear in my mind the consequences that my activity may have on the patient.

## What are the current problems with performing molecular tests on histological samples?

Pathologists must be confident on the reliability of pre-analytical procedures to avoid the so-called 'garbage in, garbage out' effect. This means that a molecular diagnosis may be distorted if the analyte (in this case, the tissue sample) is not of good quality.

The transfer, preservation and processing of human tissues is mainly based on experience rather than evidence, and recommendations are primarily designed to solve analytical problems related to tumour-specific biomarkers. The challenge is to produce standard operative pre-analytical procedures and methods for human tissue handling that can provide appropriate analytes for new molecular technologies. However, investments in these procedures are not considered a priority.

## How does vacuum packing and cooling (VPAC) prevent degradation of important DNA, RNA and protein biomarkers in the tissue samples?

Many groups have shown the damaging effect on nucleic acids and proteins from

cold ischaemia time (the time from surgical excision to fixation of the surgical specimen). As a solution to this problem, we developed VPAC. This method requires immediate vacuum sealing of excised specimens at surgical suites, followed by time-controlled transferring at 4 °C to the pathology laboratory. Such a procedure enables close monitoring of fixation time and yields fresh (not fixed) tissues for biobanking and cell culture or xenografts, while preserving nucleic acids and proteins.

## Have you developed a good working relationship with your industrial partners Milestone and A. Menarini Diagnostics?

Our working relationship with both partners has been driven by the aim to improve common practice in pathology laboratories. We work closely together, exchanging our needs with them and exploring whether they can offer interesting solutions. The development of the VPAC system was only possible because of this sharing of ideas. We have found support whenever we have asked, and applied together and received funding for a national grant sponsored by the Italian Ministry of Health.

## How do you envision the field developing in the next few years? Who will this benefit?

I hope that interest in pre-analytical anatomic pathology will increase. As a result, patients will receive better diagnosis and treatment. I also hope that researchers working on human tissues will take full advantage of the newest and most stringent molecular platforms, and therefore have greater confidence in the quality of the generated data, and move their findings towards clinical application. The dissemination of new *in vitro* diagnostic methods and technologies will lead to a significant expansion of R&D activities. Diagnostic and pharmaceutical companies will benefit from the more precise application of diagnostic markers, and national health services will be able to monitor the application of rules for certified quality controls in surgical pathology.

# Optimising **tissue sample** preservation

The Department of Medical Sciences at the **University of Turin** in Italy is leading the way in standardised processes for histopathological diagnoses, and has developed an innovative technique that can effectively preserve tissue samples

**HISTOPATHOLOGY, THE STUDY** of diseased tissue samples, is essential for the accurate diagnosis of patients. This is particularly true in the field of oncology, where histopathology is critical for the staging and grading of tumours. This insight can also determine the specific genetic cause of the cancer, enabling personalised treatment.

Indeed, as understanding of molecular biology has advanced, pathologists are increasingly using innovative molecular tests for the treatment of cancer. Several of these are already common in clinical practice, for the diagnosis of cancers based on the presence of specific gene alterations; to identify patients who would benefit from particular treatments; and predict the course of disease.

Although these new tests hold much promise, their accuracy is entirely dependent on the quality of the tissue sample, as they must be properly preserved in order for the tests to provide useful results. Known in computer science as the 'garbage in, garbage out' phenomenon, the quality of the input determines that of the output. Central to this are the processes prior to histopathology itself – the procurement, preservation and handling of tissues. These, however, are unstandardised, and can sometimes fail to preserve the integrity of sample molecules.

The Department of Medical Sciences at the University of Turin in Italy aims to provide a solution. Headed by Professor Anna Sapino, the Laboratory of Histopathology and Molecular Pathology is working to optimise and standardise key processes in pathology, including tissue preservation, transport and fixation. The lab's activity is focused on oncological diagnostics and, primarily, breast cancer. In this context, Sapino is working to ensure that biomarker analyses and molecular tests are both reproducible and reliable.

## **A TOXIC FIXER**

Current practice for the pre-analytical phases of tissue specimen management is flawed. After their surgical removal, all tissue specimens are immersed in commercial solutions of formaldehyde (formalin). This process, known as fixation, preserves the tissue at the operating theatre until it is transported to the pathology lab where it is grossed for analysis.

There are two key problems with this process. Firstly, formalin is toxic and has carcinogenic properties – as the tissues must be soaked in the solution, a large amount is needed. Moreover, as it can be inhaled, its handling (in surgical theatres, transit environments and labs) is a risk to human health. Secondly, the processes of tissue preservation, transport and fixation do not maintain the integrity of proteins (particularly of phosphoproteins) and nucleic acids, crucial diagnostic markers. While there are certain specific guidelines, such as dictating the time and form of fixation optimal for breast cancer predictive markers, there are no guidelines that pathologists can apply to all specimens. The rapid transfer of tissues to the lab is essential to preserve macromolecules but, due to the lack of guidelines, this often fails to take place.

## **SAFE AND SOUND**

To tackle these inadequacies, since 2008, Sapino and her colleagues have been collaborating with industry to introduce a new paradigm. Their alternative method, called vacuum packing and cooling (VPAC), has many advantages over traditional practices. In VPAC, specimens are vacuum packed at the surgical theatre before being cooled to 4 °C for transport to the pathology lab. This completely removes the need for formalin, a major drawback of the current method, and preserves the samples more effectively.

Comprehensive investigations into the technique suggest it is also scientifically robust. Analysing a series of over 2,000 VPAC processed samples, the team found no morphological or immunohistochemical changes to the samples – they remained



## INTELLIGENCE

### OPTIMISING SPECIMEN QUALITY FOR MOLECULAR DIAGNOSTICS

#### OBJECTIVES

To standardise processes for histopathological diagnoses and enable the effective preservation of diseased tissue samples to determine the best course of treatment for patients.

#### KEY COLLABORATOR

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#### KEY PARTNERS

A. Menarini Diagnostics

Milestone

#### FUNDING

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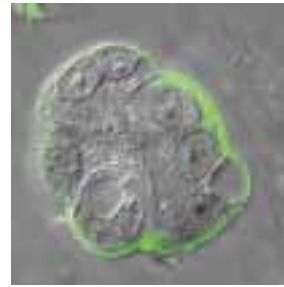
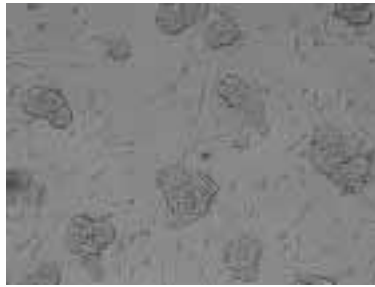
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**ANNA SAPINO** is Professor of Pathology in the School of Medicine and Head of the Pathology Unit at the University of Turin in Italy, and Chair of the Department of Laboratory Medicine at the AOU Città della Salute e della Scienza di Torino, Italy. Her research aims to innovate the surgical pathology techniques for improving the reliability of diagnosis.



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DI TORINO



Epithelial cancer cells (3D spheroid structures) and stromal cells (spindle elements) of a short-term primary culture raised from an invasive micropapillary carcinoma of the breast (EMA in green).

pathologically sound. Furthermore, the procedure was well received by those who would have to carry it out. A 2010 survey on the feasibility, compliance and quality assurance of VPAC for transferring surgical specimens was answered favourably by both surgical and pathology staff members. Based on its success, the procedure is currently being used by Sapino's lab for all surgical samples above 2 cm, of which the group has collected over 25,000 in the past five years.

A number of more recent results have also attested to VPAC's reliability. In research published just last year, the quality index for protein preservation – indicative of the quality of the sample – was shown to be significantly higher using VPAC than other procedures. Overall, short-term primary tissue cultures can be collected with an 85 per cent success rate. "These samples may represent an optimal archive of tissue biobanking," Sapino enthuses.

#### ONCOGENIC APPLICATIONS

Applying the technology to breast cancer, Sapino's area of expertise, the team demonstrated that tissues collected using VPAC are of sufficient quality for molecular testing based on gene expression profile, which can be used to predict the likelihood of the disease recurring. They focused on the HER2 oncogene, the amplification of which has a crucial role in the development and progression of aggressive forms of breast cancer. An important biomarker,

HER2 is used as a target of therapy for approximately 15 per cent of patients.

Sapino's team approached the gene with an interesting hypothesis: "In the case of polysomy (additional copies) of chromosome 17, where HER2 is located, it was thought the increase in gene copy number was not related to a true amplification, making the patient ineligible for treatment," Sapino explains. Working with world-leading molecular pathologists to apply microarray-based comparative genomic hybridisation to specimens, the researchers showed that indeed, chromosome 17 polysomy is a rare event, and does not influence analysis of HER2 copy number. Their results, published in 2009, were later incorporated into the Association of Clinical Oncologist (ASCO) and the College of American Pathologists (CAP) recommendations for HER2 testing. Their findings even informed a new algorithm for the assessment of HER2 status, ensuring that all patients have access to anti-HER2 therapy.

In recognition of the high quality results produced to date, VPAC has already been translated into clinical practice. It is fully in use in Sapino's lab, and has also entered the Italian guidelines for tissue handling in breast surgical pathology. Internationally speaking, institutions including the Henry Ford Hospital in the US have begun to use VPAC. By increasing the quality of tissue samples, the group is ensuring more accurate diagnoses are made, ultimately enhancing patient care.



#### INDUSTRIAL TIES

Sapino's group is collaborating with two companies to improve the use of tissues for molecular diagnostics. Both work on the development of new technologies for tissue preservation and fixation, as well as new methods to image histological sections and analyse biomarkers

##### A. Menarini Diagnostics

A leading Italian pharmaceutical company, A. Menarini Diagnostics was established to respond to the growing need for diagnostic tests and preventive medicine. The company now operates in a number of *in vitro* diagnostics fields including clinical chemistry, immunology and histology.

##### Milestone

Milestone specialises in advanced microwave instrumentation for analytical and organic chemistry labs, with a separate medical division to transfer its expertise in microwave technology to histopathology. It was the first company to design a fully automated high-throughput microwave processor – today, the most widely used microwave processors in the field.