Critical Steps in Tissue Processing in Histopathology

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Received: September 27, 2011 Revised: November 23, 2011 Accepted: November 27, 2011

Abstract: Histopathological diagnosis using Formalin-Fixed Paraffin Embedded (FFPE) tissues is essential for the prognostic and therapeutic management of cancer patients. Pathologists are being confronted with increasing demands, from both clinicians and patients, to provide immunophenotypic and gene expression data from FFPE tissues to allow the planning of personalized therapeutic regimens. Recent improvements in the protocols for pre-analysis processing of pathological tissues aim to better preserve cellular details and to conserve antigens and nucleic acid sequences. These developments have been recently patented. The international protocol for the transporting of surgical specimens from the surgical theatre to the pathology department is to immerse the specimen in formalin. The alternative method of sealing the specimens into bags under a vacuum and then cooling is a well-accepted and environmentally safe procedure that overcomes the many drawbacks linked to transfer in formalin. Importantly, RNA is notoriously poorly preserved in FFPE tissue. Due to this, successful procedures for the extraction of genetic information from archival tissues have been the object of several studies and patents. Novel molecular approaches for RT-qPCR and gene array analysis on FFPE tissues are presented here. Moreover, a major advance is reported in this study, the observation that tissue fixation in cold conditions allows a much better preservation of nucleic acid sequences.

Keywords: Breast cancer, fixation, formaldehyde, formalin, genes, histopathology, neoplasms, nucleic acids, pathology, RNA, RT-PCR, sealing, tissues, tissues banks, under-vacuum.

INTRODUCTION

Histopathological diagnosis remains an integral component of the prognostic predictions and therapeutic planning of diseases. The field has been gaining further interest in recent years with the advent of personalized therapies for different tumor entities. Previously, diagnosis was only based on morphological features. Therefore, histopathological patterns, comprehensive of ultrastructural features, were the main basis for disease classification. The use of histochemical and immunohistochemical techniques has allowed pathologists to develop more precise, reliable and reproducible disease classifications. These techniques allow the pathologist to complement morphology with information regarding protein (antigen) expression and distribution.

Since the year 2000, pathologists have made critical steps forward in the knowledge of the pathogenesis and genetic profiles of several cancer types and this has made significant impact on prospects for both cancer prevention and the use of novel personalized therapeutic regimens. Progressively attention has moved toward gene analysis as a method of examining both origin and differentiation of various tumor types. Molecular analysis is thus emerging as an essential technique to complement conventional histopathology. This is reflected by the progressive evolution of the WHO Classification report outlined in the "Blue Books". This report initially only dealt with histological features, but more recent editions use the results of genetic analysis to complement, but never substitute for, the morphological characterization [1-3].

The consequence of this improvement is that cancer diagnosis for individual patients has become more complex and molecular tests have become mandatory, for example, for the identification of gene mutations responsible for familiar hereditary tumors of endocrine organs (MEN 1 and MEN 2 Syndromes) [4] or the assessment of microsatellite instability for the identification of carriers with increased risk for Lynch Syndrome [5-7]. Nowadays, morphological diagnoses are not sufficient for planning personalized therapies that need, for example, the detection of chromosomal translocations and aberrations in sarcomas and brain tumors [8-12]. the evaluation of mutations of EGFR and K-RAS genes in lung and colorectal adenocarcinomas [13-16], of cKIT and PDGFRA genes in cases of gastro-intestinal stromal tumors (GISTs) [17-19] and of BRAF and NRAS genes in melanomas [20, 21], and last but not least the evaluation of the status of the HER2 for breast cancer [22, 23].

These tests require a proper preservation of tissues, so that it can be analyzed in parallel for cell and tissue structural arrangement, protein (antigen) distribution and used for gene sequencing. The first, as well as the most crucial step of tissue processing in histopathology is the proper collection of the biological material. Microscopic diagnosis is strictly dependent on the technical preparation, the goal of which is obtaining thin sections optimal for analysis under the microscope, while at the same time preserving the integrity of both the structure and biology, particularly of the proteins and

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