

# Applicability of Under Vacuum Fresh Tissue Sealing and Cooling to Omics Analysis of Tumor Tissues

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**Context:** Biobanks of frozen human normal and malignant tissues represent a valuable source for “omics” analysis in translational cancer research and molecular pathology. However, the success of molecular and cellular analysis strongly relies on the collection, handling, storage procedures, and quality control of fresh human tissue samples.

**Objective:** We tested whether under vacuum storage (UVS) effectively preserves tissues during the time between surgery and storage for “omics” analyses.

**Design:** Normal and matched tumor specimens, obtained from 16 breast, colon, or lung cancer patients and 5 independent mesenchymal tumors, were dissected within 20 minutes from surgical excision and divided in three to five aliquots; for each tissue sample, one aliquot was snap-frozen in liquid nitrogen (defined as baseline or T0 samples), and the other portions were sealed into plastic bags and kept at 4°C for 1, 24, 48, or 72 hours under vacuum and then frozen. The tissue and molecular preservation under vacuum was evaluated over time in terms of histomorphology, transcription (Illumina microarrays), protein (surface-enhanced laser desorption/ionization-time of flight/mass spectrometry and Western blot), and metabolic profile (nuclear magnetic resonance spectroscopy).

**Results:** Tissue morphology, Mib-1, and vimentin immunostaining were preserved over time without signs of tissue degradation. Principal variance component analysis showed that time of storage had a minimal effect on gene expression or the proteome, but affected the preservation of some metabolites to a greater extent. UVS did not impact the RNA and protein integrity or specific phosphorylation sites on mTOR and STAT3. Measurement of metabolites revealed pronounced changes after 1 hour of storage.

**Conclusions:** Our results show that UVS can preserve tissue specimens for histological, transcriptomic, and proteomic examinations up to 48 hours and possibly longer, whereas it has limitations for metabolomic applications.

## Introduction

RECENT PROGRESS IN health research toward realizing the goal of personalized medicine,<sup>1</sup> together with advances in new technological platforms and the “omics” revolution,<sup>2</sup> has opened new opportunities to derive important information about disease mechanisms directly from tissue samples.<sup>3</sup> This has been the case with research focused on human tumors; thus, efforts have increased for the establishment of biobanks of fresh tissues.<sup>4</sup> The purpose of tissue banks is to enhance the quality and the speed of both basic and translational research,<sup>2</sup> providing unique resources for studying molecular changes in the *in situ* environment of cancer.

On this basis it could be expected that comprehensive genomic, proteomic, and even metabolic profiling repre-

sentative of the biological complexities of health and disease might replace single diagnostic biomarkers. In fact, with the introduction of new genomic technologies, such as tissue-based RNA microarrays, patterns of gene expression able to stratify tumors according to their molecular features and predict clinical outcomes have been discovered in various cancer types.<sup>5</sup>

Thus, storage of tissues with effective preservation of morphology, proteins, nucleic acids, and metabolites for research and diagnostic purposes is the main goal of human tissue biobanks.<sup>6</sup> As a consequence, collecting surgical specimens that can be used for these analyses has become a mandatory issue for assessing the correlation between clinical features and molecular data, in the majority of current clinical trials.<sup>7</sup> However, variability in tissue handling and processing

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