

## Talk Abstract

**53rd Symposium of the Society for Histochemistry  
Munich, Germany, October 12-15, 2011**

### **Evaluation of PAXgene-fixed, Paraffin-embedded Tissues for Morphological and Molecular Analysis**

S. Gündisch<sup>1,6</sup>, C. Schott<sup>1,6</sup>, B. Reischauer<sup>1,6</sup>, S. Meding<sup>2</sup>, R. Langer<sup>1,6</sup>, M. Kap<sup>4,6</sup>, C. Viertler<sup>5,6</sup>, U. Ferch<sup>3</sup>, P. Riegman<sup>4,6</sup>, K. Zatloukal<sup>5,6</sup>, A. Walch<sup>2</sup>, KF Becker<sup>1,6</sup>

<sup>1</sup>Institute of Pathology, Technische Universität München, Munich, Germany

<sup>2</sup>Institute of Pathology, Helmholtz Center Munich, Neuherberg, Germany

<sup>3</sup>Third Medical Department, Technische Universität München, Munich, Germany

<sup>4</sup>Department of Pathology, Josephine Nefkens Institute, Rotterdam, The Netherlands

<sup>5</sup>Institute of Pathology, Medical University of Graz, Graz, Austria

<sup>6</sup>The SPIDIA Consortium, [www.spidia.eu](http://www.spidia.eu)

**Aims:** For molecular diagnostics and personalized medicine protein biomarkers need to be precisely measured in clinical tissue samples. In formalin-fixed and paraffin embedded (FFPE) tissues protein analysis is still challenging. Within the European project SPIDIA we evaluated the novel formalin-free tissue fixative “PAXgene Tissue System” for better integration of morphological and molecular analysis, focussing on protein approaches.

**Methods:** Different murine and human tissue samples were either snap-frozen, fixed with the novel tissue fixative, PAXgene tissue fixation and stabilization reagents, or with formalin before paraffin-embedding. Proteins were analyzed by Coomassie staining, two-dimensional gel electrophoresis, Western blotting, reverse phase protein microarrays (RPPA) and matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). The preservation of the phosphoproteome was investigated in a large-scale comparative study with 16 different non-malignant and 4 different malignant tissue entities with 11 phosphorylation-specific antibodies by Western blot. Morphology and immunohistochemistry were evaluated and RNA quality was assessed by PCR amplification assays.

**Results:** We were successful in extraction of non-degraded and immunoreactive proteins from PAXgene-fixed tissue specimens. We analyzed for example E-cadherin, Hsp70 and beta-actin and phosphorylated proteins, including p-Akt, p-Erk-1/2, and p-NFkB. Recovered proteins showed very similar properties when compared to cryopreserved samples by Western blotting or RPPA and were superior to proteins from FFPE samples. Furthermore, the spectra of MALDI-IMS analysis were similar to cryopreserved samples which were visualized by insulin and glucagon expression in pancreatic tissue. Finally, morphology was comparable to FFPE samples whereas RNA was far better preserved in PAXgene-fixed samples.

**Conclusion:** The PAXgene Tissue System preserves not only the proteome and most importantly the phosphoproteome of a tissue sample but as well morphology and nucleic acids. Thus, it has great potential to serve as a novel multimodal fixative for modern pathology, enabling widespread biomarker studies on clinical tissue samples.