**Evaluation of the PAXgene® Tissue System: Preservation of Morphology and Gene Expression in Human Melanoma**

Peter Hesse, Ralf Wyrich, Thorsten Voss, Lynne Rainen and Daniel Grölz,
PreAnalytiX GmbH, Hombrechtikon, CH

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**Introduction**

PAXgene Tissue System
• Formalin-free tissue fixation method
• For simultaneous preservation of tissue morphology and biomolecules
• Allows analysis of biomarkers from the same sample used for histopathology
• Two reagent system for tissue fixation and stabilization
• Consists of the PAXgene Tissue Container, PAXgene Tissue Kits and supplementary protocols (www.preanalytiix.com)
• Currently under evaluation by the EU FP7 funded project SPIDIA

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**Materials and Methods**

- **Staining:** Hematoxylin & Eosin, in-situ hybridization, immunohistochemical staining for Ki-67 and S100
- **Probe purification:** PAXgene Tissue RNA (PreAnalytiX), negative tissue control (PreAnalytiX)
- **RT-qPCR array**
  - TaqMan Array: Human Endogenous Control with 32 control genes, plated in triplicates in a 96-well format
  - Two Step RT-PCR with 2 µg RNA per plate
  - R²: coefficient of determination

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**Results**

Figure 1: Staining

- A: Case 1
  - FFPE
  - H&E

- B: Case 2
  - FFPE
  - H&E

- B: Case 3
  - FFPE
  - H&E

- Antibodies: Ki-67 clone MIB-1 (Dako M7240, 1:75 dilution); S100 polyclonal rabbit (Dako Z0311, 1:400 dilution)
- Epitope retrieval: FFPE 20 min at 98°C in citrate buffer pH6, PFPE 10 min at 70°C (Ki-67) or at 98°C (S100) in Tris/EDTA

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**Scatterplots for comparison of Ct values**

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**Conclusions**

Stabilization of human melanoma tissue with PAXgene Tissue:
• H&E morphology: well preserved, comparable to FFPE
• Antigenicity: for Ki-67 and S100 comparable to FFPE
• With regard to location, staining intensity and number of stained cells
• Gene expression profile:
  - high concordance between RNA from PFPPE and snap frozen tissue
  - low concordance between RNA from PFPE and FFPE
• Small, noncoding RNA profiles:
  - high concordance between RNA/miRNA from PFPE and snap frozen tissue
  - high concordance between RNA/miRNA from PFPE and FFPE

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