Technological Advances in Molecular Pathology:

A Journey into the Archives

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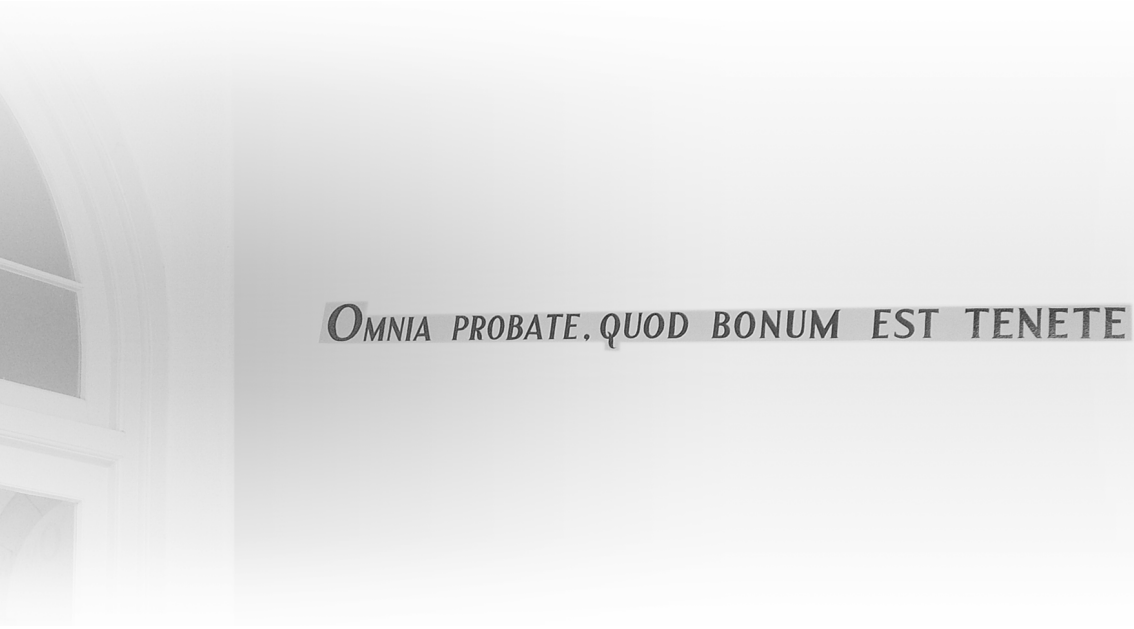

Entrance of the “old” Pathology building. Wassenaarseweg, Leiden

Aan Marianne

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Aims and outline

Pathology laboratories throughout the world have compiled large archives of unique collections of tissue specimens. These tissue samples are used for patient diagnostics and research. Novel molecular insights into alterations in normal cellular function have led to the identification of targets for innovative therapies. Testing for biomarkers combined with molecular pathology has created the potential for “personalized medicine” and improved diagnosis, treatment and prognosis. New technologies for molecular analysis in molecular tumor diagnostics and research must be developed and implemented to keep pace with the latest insights, resulting in a constant cycle of change. Such translational research can only progress if patient material can be accessed from the archives for further study. The resulting new insights and strategies will eventually be implemented in patient care.

This thesis describes three important issues in this cycle of change with a focus on molecular pathology.
First, due to advances in the treatment of cancer, the amount of patient material that is available for diagnostics and research is decreasing, while the number of requests for diagnostics is rapidly increasing. Early diagnosis and the increasing application of neoadjuvant therapies are primarily responsible for this trend. The latter is, without a doubt, beneficial to the patient but makes the (molecular) diagnostics of the material increasingly challenging.
Before molecular techniques can be applied for diagnostics or research, nucleic acids must be extracted from the archived tissue. Because the tissue is fixed in formalin and embedded in paraffin, the nucleic acids are crosslinked and fragmented and, consequently, of poor quality. Therefore, DNA isolation procedures must be improved, and if the amount of DNA remains too low for biomarker testing, analysis methods such as whole genome amplification should be considered.
Second, new state-of-the-art technologies must be developed constantly. Methods should be validated and implemented and be applicable for use with the small amounts of DNA isolated from tissue that is heavily degraded in the embedding process.
The third focus of this thesis is the use of bio-informatics approaches. For the genomic data analysis of tumors in some applications, only limited analysis software is available. In a research environment, new tools must be developed, applied, or adapted for the analysis of the acquired data. After validation, these tools can be implemented in the daily routine of molecular tumor diagnostics.

In **chapter 1**, developments in pathology over the centuries and molecular technology and pathology in recent decades are summarized in a historical perspective. Some important genes and interesting genomic phenomena are highlighted in the context of their clinical implications. Guided by the description of a DNA analysis pipeline, the three different foci of this thesis, pre-analysis

technologies, technological advances and analysis strategies, are further introduced. **Chapter 2** demonstrates how DNA can be isolated from formalin-fixed, paraffin-embedded (FFPE) material and compares two different techniques: a manual method and a fully automated DNA isolation method. **Chapters 3 and 4** describe multiplex ligation-dependent probe amplification (MLPA) as an assay for the detection of multiple chromosomal deletions in tumor tissue in a single experiment. We developed and validated a MLPA-based assay to identify chromosomal losses in formalin-fixed and paraffin-embedded oligodendroglial tumors. To assure a reliable workflow for this technology, a data management system, MLPAInter, was developed to interpret the MLPA data stream. **Chapter 5** reveals how limited amounts of DNA can be amplified in a whole-genome amplification (WGA) process in which a two-step mutation screening protocol is applied. First, a high-resolution melting analysis (HRM) is used as a prescreening method for samples harboring mutations, and direct Sanger sequencing is then employed for the final diagnosis of the mutations. The *KRAS* gene was used as a model system because the accurate detection of *KRAS* mutations is critical for the molecular diagnosis of cancer and may guide proper treatment selection. In **chapter 6**, the reliability with which allele-specific quantitative real-time PCR with hydrolysis probes could be performed on fine-needle aspirates from non-small-cell lung cancer (NSCLC) patients was studied by comparing the results with histological material from the same patients. Finally, future directions and concluding remarks are presented in **chapter 7**.