Tissue quality for molecular biology and pathology

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Molecular biology and pathology

Molecular biology and pathology requires high-quality, intact genomic DNA, mRNA, and proteins

- DNA assays (conventional and real-time PCR: DNA detection and quantification, in situ hybridisation)
- RNA assays (RT-PCR: gene expression studies, micro-arrays: expression profiling, in situ hybridisation)
- Proteome analysis (immunohistochemistry)

Selection of material is essential

- Selection biased (false negative)
- Role of imaging

Molecular biology and pathology

- Use tissue for molecular diagnostics in clinical setting
  - E.g. FISH Her2 breast cancer
- Use tissue in research
  - Epidemiology
  - Biomarker studies
  - Studies on molecular mechanisms of disease
- Fresh/frozen tissue versus archival tissue

Molecular biology and pathology

Dual-colour FISH staining used to determine HER2 status as part of breast cancer diagnosis.

Molecular biology and pathology

HPV detection and laser capture microscopy technology in cervical cancer research: which HPV type caused the lesion? (Wim Quint, HPV today)

A cervical biopsy stained for p16 and showing a complex lesion with areas of CIN2 and CIN1/2. Some areas of CIN2 show diffuse basal p16 staining. LCM/PCR shows that HPV31 and HPV51 are present in separate areas of CIN. Some areas with HPV31 and CIN2 express p16 strongly, whereas HPV51 areas do not.

Morphology-based versus in vitro (lump) testing

- Morphology-based testing
  - Morphological control of tissue (presence of tumour, necrosis, validation, ….)
    - Immunohistochemistry
    - FISH
- Lump testing
  - Material is used for extraction, therefore not available for morphological assessment
  - Value of negative result
    - DNA/RNA extraction
    - Array testing

Molecular biology and pathology

- Fresh or frozen tissue: best source for analysis of nucleic acids and proteins

  Disadvantages frozen tissue
  - Inferior morphology (compared to FFPE tissue)
  - Infectious agents keep biohazard potential
  - High costs
- Archives of pathology labs/biobanks: formalin-fixed, paraffin-embedded tissue

Factors influencing tissue quality

- Optimized tissue preservation and fixation is critical for tissue-based research
- Long-term storage of fresh or frozen tissue is often unfeasible
- Factors that impact tissue quality
  - Prior biopsy
  - Intra-operative hypoxia and ischemia
  - Time to fixation
  - Type, concentration and adequacy of fixative
  - Method of paraffin block preparation

Frozen tissue

- Snap-freezing without cryoprotection at <-78° C: extraction of genomic DNA, mRNA, or protein
- Freezing at <-78° C with a cryoprotectant: preserve tissue architecture and cytological features for immunohistochemistry and in situ hybridization
- Long-term storage of the frozen tissue is recommended at <-140° C in liquid nitrogen freezer

Fixation with formalin

- Formalin fixation = tissue storage for years
  - Low cost
  - Preservation of morphological details
  - Biosecurity
- Mode of action

  Reversible cross-linking between proteins by the formation of reversible methylol derivates, Schiff-bases, and stable methylene bridges

  Maintain structural integrity and prevent enzymatic degradation (autolysis and heterolysis)

  Microbicidal properties: infectious tissue can be stored

Fixation and proteins

- Formalin induces protein cross-linking
- Multiple antibody-antigen combinations do not work in tissues fixed in 10% NBF
- Loss of immunorecognition is sometimes an issue for Ki67/MIB, estrogen receptor alpha, progesterone receptor, and partial for Bcl-2
  - Antigen retrieval
  - Tuning of the antibody to work on formalin-fixed epitope
- Heat-induced reversal of cross-linking in combination with antigen-retrieval buffers

Fixation and nucleic acids

Formalin fixation induces degradation and chemical modification of the nucleic acids

⇒ Extraction using standard techniques fails

- Impact on NA quality (fragmentation)
- Impact on NA quantity (cross-linking NA and proteins)

⇒ Amplication fails

- No amplification of long PCR targets (fragmentation)
- Loss polyA tail of RNA: no annealing poly(dT)primers during RT

Fixation and nucleic acids

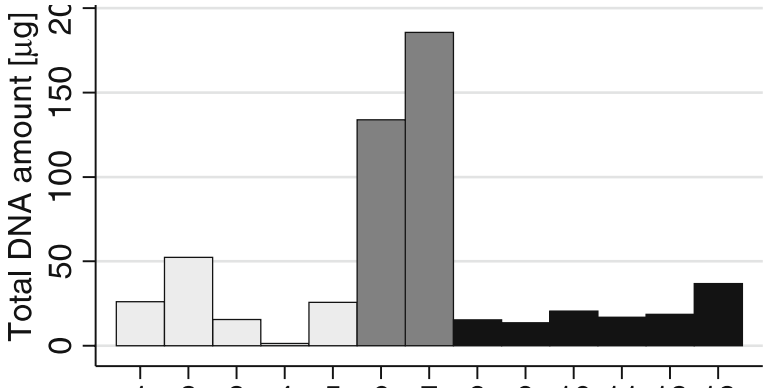
- Optimise NA isolation techniques
  - DNA extraction
    - Deparaffination and digestion of FFPE tissue
    - Reduce thickness of paraffin section
    - Published protocols for DNA extraction from FFPE tissue have been compared: Bonin et al. Virchows Arch, 2010
  - RNA extraction
    - mRNA analysis often more focused on quantification: published protocols to limit loss of RNA amounts

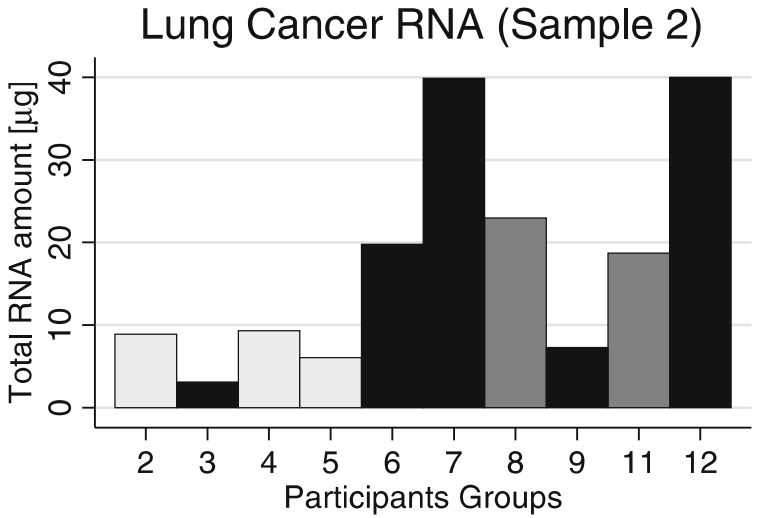
Comparison of nucleic acid extraction protocols

- DNA extraction
  - Robust and often good
    - PCR products of 200-250bp
    - Tissue dependent
    - QA is essential
    - Silica-based absorption columns are the most accurate
- RNA
  - Chromatography-based columns are the best
    - Specific paraffin extraction kits from Qiagen and Roche

Bonin et al, Virchow archiv 2010: 309-317

DNA/RNA extraction from FFPE tissue





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Fixation and nucleic acids

- Optimise (RT-)PCR
  - Short PCR target
  - Normalization: use of reference genes
- Use alternative fixatives
  - Alcohols: no cross-linking of NA and proteins
  - Commercial fixatives, e.g. UMFIX: preserve NA at RT and allows IHC
  - Other features, such as stability, cost, disinfectant properties and tissue preservation inferior to formalin

Conclusion

New technologies!

- high-throughput assessment of biomarkers feasible
- extended range of assays that can be done on fixed tissues
- Tissue selection remains vital
  - Lack of morphological control
  - Importance of high quality imaging and biopsy-taking