Objective

To evaluate morphology and nucleic acids in FFPE and PFPE tissue, stored for four years at different temperatures.

Methods

- Tissue fixation, processing, and storage:
  - Rat liver, kidney, spleen, lung and intestine were grossed immediately after resection. From each organ, rimmed pairs of samples, identical in size, were placed into tissue cassettes and fixed at room temperature in either the PAXgene Tissue Container (PreAnalytiX) or with neutral buffered formalin (Sigma).
  - PAXgene: 4 h fixation, 24 h stabilization
  - Formalin: 24 h fixation
  - All samples were processed on a TP1020 with 70%, 80%, 90%, 95% (3x) ethanol, isopropanol (2x), xylene (2x), paraffin (3x, Paraplast-XTRA) at 56°C.
- RNA extraction and analysis:
  - RNA was extracted from 3 x 10 μm sections of PFPE tissue with PAXgene Tissue RNA kit (PreAnalytiX) and from FFPE tissue with RNeasy FFPE kit (QIAGEN) and eluted in 30 μl elution buffer supplied in the kits.
  - Yield was determined with a Nanodrop Spectrophotometer and integrity on agarose gel electrophoresis.
  - Real time RT-PCR was conducted with a Rotor-Gene Q (QIAGEN) in one-step RT-PCR reactions using the Quantitect SYBR® Green RT-PCR kit (QIAGEN) with assay volumes of 25 μl with 2 μl eluates diluted 1:10 with water.
- DNA extraction and analysis:
  - DNA was extracted from 3 x 10μm sections of PFPE with PAXgene Tissue DNA kit (PreAnalytiX) and from FFPE with QIAamp FFPE kit (QIAGEN) and eluted in 30 μl elution buffer supplied in the kits.
  - Yield was determined with a Nanodrop Spectrophotometer and integrity on agarose gel electrophoresis.
- Real time PCR was conducted with a Rotor-Gene Q (QIAGEN) in one step PCR reactions using the Quantitect SYBR® Green PCR kit (QIAGEN) with assay volumes of 25 μl with 2 μl eluates diluted 1:10 with water.

Results

- Nucleic acid degradation occurs in FFPE and PFPE tissue at ambient temperatures (Fig. 1 and 4).
- No nucleic acid degradation was observed in frozen FFPE and PFPE tissue (Fig. 1 and 4).
- There were advantages in PCR performance with regard to lower Ct values and less failures for RNA and DNA from FFPE versus FFPE samples regardless of storage temperature (Fig. 2 and 5).
- RNA and DNA from frozen FFPE samples show advantages in PCR as compared to RNA and DNA from samples stored at ambient temperatures (Fig. 3 and 5).
- H&E histology was preserved in PFPE samples stored up to four years (Fig. 6 – data shown for storage at 22°C only).

Conclusion

- After storage for four years, tissue morphology is preserved in archived FFPE tissue.
- After storage for four years, nucleic acids from FFPE samples perform better in PCR and RT-PCR than nucleic acids from PFPE samples.

Fig. 1: RNA integrity values for RNA isolated from rat FFPE and FFPE tissue stored for 4 years at 22°C, 4°C, -20°C or -80°C. Average values from liver, kidney, spleen, lung and intestine with three replicates each.

Fig. 2: Performance differences in SYBR Green real time RT-PCR between RNA from PFPE and FFPE tissue. A: Assay design for amplification of rat beta-actin gene with one common forward primer and different reverse primers to generate amplicon lengths from 109 to 610 base pair. B: ΔCt values calculated by subtraction of C_t values obtained with RNA from PFPE blocks stored for four years at 22°C, 4°C, -20°C or -80°C from C_t values obtained with RNA from corresponding FFPE samples; average values and standard deviations from triplicate extractions of five different tissue types. C: Example for amplification plot and melting curve analysis from rat liver FFPE and FFPE tissue.

Fig. 3: Performance in SYBR Green real time RT-PCR. A: Number of samples [%] positive for each assay from Fig. 2. B: Detailed analysis of PCR failures [%] for FFPE samples stored at 22°C, 4°C, -20°C or -80°C.

Fig. 4: DNA integrity analysis from PFPE and FFPE tissue by agarose gel electrophoresis. PFPE and FFPE blocks were stored prior to DNA extraction for 4 years at 22°C (1), 4°C (2), -20°C (3) and -80°C (4). 200 ng genomic DNA was separated on 0.8% TAE agarose gel. Examples are shown for DNA from rat kidney (A) and intestine (B).

Fig. 5: Performance differences between DNA from PFPE and FFPE tissue in four different SYBR Green real time PCR assays for amplification of 271, 523, 650 or 747 bp of rat beta-actin gene. A: ΔCt values calculated by subtraction of C_t values obtained with DNA from FFPE blocks stored for 4 years at 22°C, 4°C, -20°C or -80°C from C_t values obtained with DNA from corresponding FFPE samples; average values and standard deviations from triplicate extractions of five different tissue types. B: Example for amplification plot and melting curve analysis from rat spleen PFPE and FFPE tissue. C: Number of samples [%] stored at different temperatures negative for the 747 bp assay.

Fig. 6: Hematoxylin & Eosin stained sections of FFPE and PFPE blocks of rat tissue stored for 4 years at 22°C.

A: Overview of whole section with identical sizes for PFPE and FFPE samples from each organ. B: Morphology of rat liver (a), kidney (b), spleen (c), lung (d) and intestine (e); original magnification x400.

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