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Technical Note
PAXgene® Tissue System

Influence of formalin contamination during processing of PAXgene Tissue fixed, paraffin-embedded tissue (PFPE) on RNA yield, integrity, and performance in quantitative RT-PCR

Study Design

Rat (*Rattus norvegicus*) tissue from liver and intestine was grossed into samples sized approximately 4 x 10 x 10 mm and placed into standard histocasettes for fixation with PAXgene Tissue Fix for 4 hours and stabilization in PAXgene Tissue Stabilizer for 24 hours. Before processing, samples were incubated in 70% ethanol, either undiluted or mixed with 1% (v/v) neutral buffered formalin (NBF) for up to 12 hours. Incubation times in 70% ethanol with 1% (v/v) NBF were 0, 2, 6, and 12 hours (Table 1). Processing and paraffin embedding were performed by following the recommendations in the *PAXgene Tissue Container Product Circular*.

Table 1. Incubation times in 70% ethanol with or without NBF

Sample	70% ethanol	70% ethanol with 1% (v/v) NBF
Reference	12 hours	–
2h 1% NBF	10 hours	2 hours
6h 1% NBF	6 hours	6 hours
12h 1% NBF	–	12 hours

RNA was purified from four PFPE (PAXgene Tissue fixed, paraffin embedded) tissue sections with 10 µm thickness using the PAXgene Tissue RNA Kit. All extractions were done in triplicates. RNA yield was analyzed by measuring the

absorbance at 260 nm. The RNA integrity was analyzed on the Agilent® bioanalyzer with the RNA 6000 nano assay. Performance in quantitative real time RT-PCR was analyzed with a TaqMan® Primer/Probe assay for amplification of a 294 bp fragment of the β-actin mRNA.

Results

RNA yield from 4 x 10 µm sections of PFPE reference samples, incubated in 70% ethanol without NBF, was 7.8 µg for liver and 5.4 µg for intestine. Yield decreased with increasing length of time that tissue was incubated in ethanol contaminated with NBF (Figure 1). In liver the decrease in yield was more prominent compared to intestine, with a reduction after 12 hours to 0.5 µg or only 6% of the yield from the reference samples.

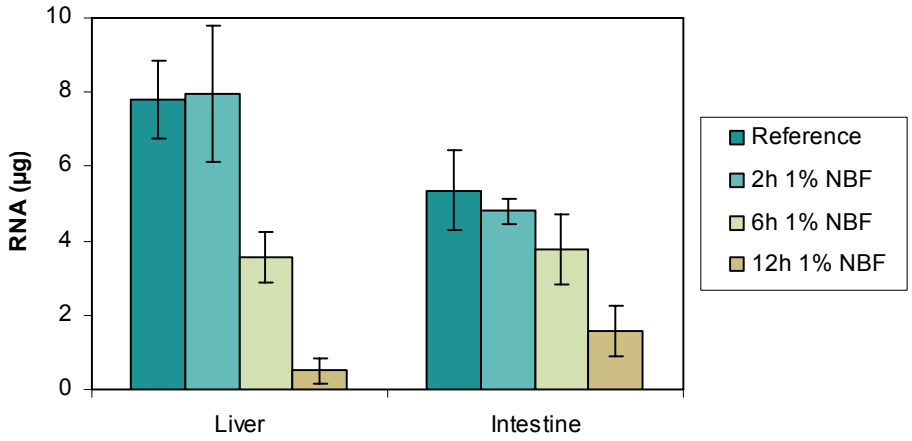

Figure 1. RNA yield from samples incubated for up to 12 hours in 70% ethanol contaminated with 1% NBF before processing. Spectrophometric analysis of RNA yield with a NanoDrop® instrument. RNA was purified from 4 sections (10 µm each) of PFPE with the PAXgene Tissue RNA Kit. Samples were incubated for 2, 6, and 12 hours in 70% ethanol mixed with 1% (v/v) NBF before processing and paraffin embedding; **Reference**: Incubation for 12 hours in 70% ethanol without NBF.

RNA integrity was less affected by incubation in NBF contaminated with ethanol. RIN values stayed at a high 7.5 in the case of liver and 8 in the case of intestine and dropped down to around 6 after 12 hours incubation in ethanol and NBF (Figure 2A). However, in the electropherogram and gel generated by the bioanalyzer the difference between incubation for 12 hours in ethanol without or with NBF contamination became obvious (Figure 2B).

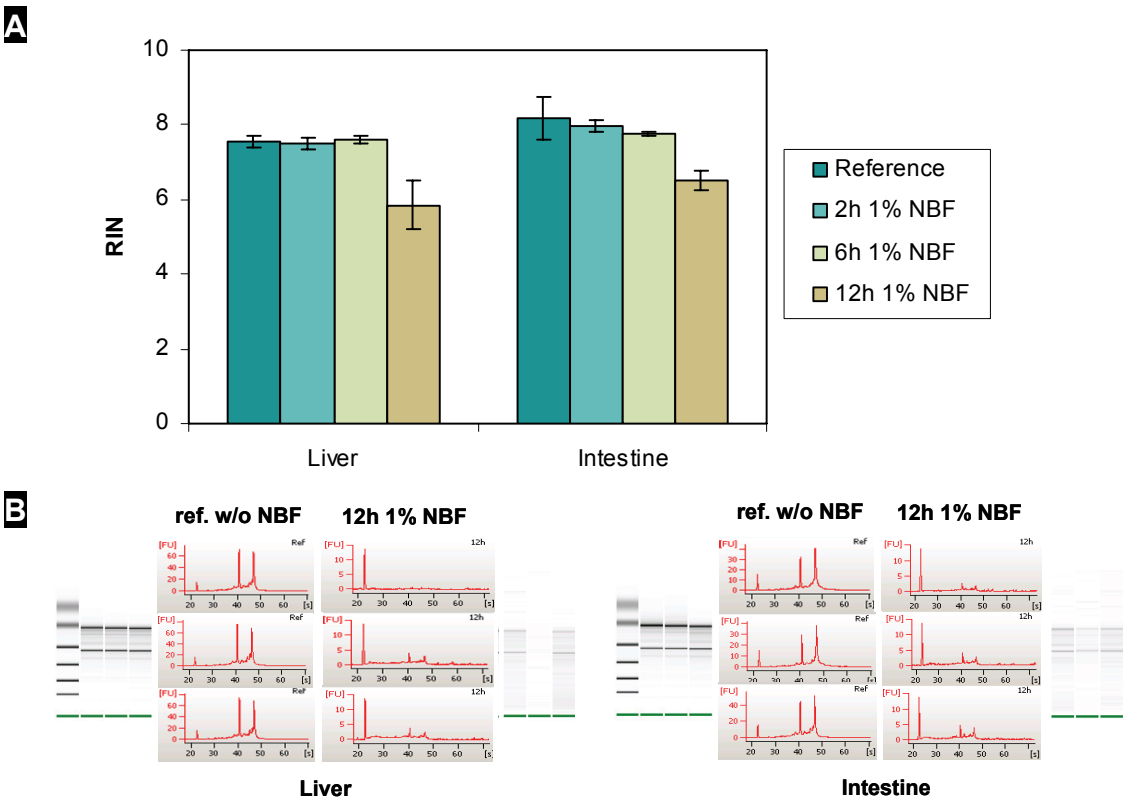

Figure 2. RNA integrity from samples incubated for up to 12 hours in 70% ethanol contaminated with 1% NBF before processing. RNA integrity analysis with the Agilent bioanalyzer; **A** RIN values (RNA integrity numbers); **Reference**: incubation for 12 hours in 70% ethanol without NBF; **B** Electropherogram and gel; samples were incubated for 2, 6, and 12 hours in 70% ethanol mixed with 1% (v/v) NBF before processing and paraffin embedding. **ref w/o NBF**: incubation for 12 hours in 70% ethanol without NBF.

In addition to lower yield, the performance of the RNA in downstream applications became worse. When 10 ng of RNA was amplified in a quantitative real time one step RT-PCR assay, RNA from the sample incubated 12 hours in 70% ethanol with 1% NBF reached, on average, a 3.7 higher C_T value in the case of liver and a 2.9 higher C_T value in the case of intestine compared to RNA from samples incubated in pure 70% ethanol (Figure 3).

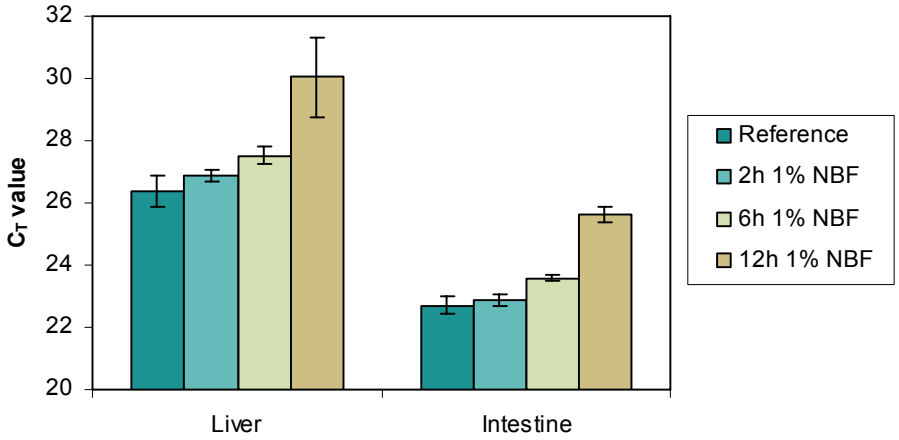

Figure 3. β-Actin real time RT-PCR with 10 ng RNA from samples incubated for up to 12 hours in 70% ethanol contaminated with 1% NBF before processing. Gene expression analysis of β-actin by quantitative real time RT-PCR. Triplicate RNA extractions from PFPE were amplified in duplicate using 10 ng RNA with the QIAGEN® QuantiTect® Probe RT-PCR Kit. Samples were incubated for 2, 6, and 12 hours in 70% ethanol mixed with 1% (v/v) NBF before processing and paraffin embedding; **Reference**: incubation for 12 hours in 70% ethanol without NBF.

Conclusion

When processing specimens fixed in PAXgene Tissue Containers, **do not use reagents contaminated with formalin**. Even trace amounts of formalin in the alcohol or other reagents used for sample processing can lead to a significant reduction in RNA yield and poor performance in quantitative RT-PCR.

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PreAnalytiX GmbH, 8634 Hombrechtikon, CH.