

BRIEF METHOD

Preservation of Tissue RNA in Normal Saline

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After decades of mostly morphological analysis, the field of pathology is rapidly incorporating a vast array of molecular assays in the diagnosis of various diseases. The analysis of messenger RNA is becoming increasingly important not only for the diagnosis, but also for prediction of outcome and as a potential target of treatment (Ross, 1999). However, current tissue processing methods in histology laboratories use formalin fixation that yields poor quality RNA because of degradation (Anderson et al, 1997). At the present time, high quality RNA is extracted only from fresh tissue (Fejzo and Slamon, 2001). Because the availability of fresh tissue is limited and special handling and storage is required, research studies have to be planned prospectively. In many instances, there may be no fresh tissue available for molecular studies. In a search for a molecular-friendly tissue preservative/fixative, we tested a number of commonly used histology reagents and assessed their effect on RNA integrity.

Mouse liver, instead of human tissue, was chosen for complete control of its freshness. RNA was extracted from ~50 mg of tissue immersed in 5 ml of various reagents using Trizol reagent (GIBCOBRL, Gaithersburg, Maryland) following manufacturer's instruction. We chose to test the effects of 10% phosphate buffered formalin, 100% ethanol, and xylene, as these are the most commonly used reagents for tissue fixation/preservation and routine processing (Carson, 1997). RNALater (Ambion, Austin, Texas)-incubated tissue and fresh snap-frozen tissue were also included as positive controls. In addition, we immersed tissue in normal saline solution (0.9% sodium chloride) as our negative control because we expected it to yield degraded RNA. Tissue was incubated at room temperature in each solution for 15 minutes, 1 hour, 4 hours, and 24 hours, to simulate routine tissue handling procedures. Following extraction, RNA was run

on standard 1% agarose gel under denaturing conditions with ethidium bromide (10 µg/ml). Quality of RNA was judged by the ratio of 28S and 18S ribosomal bands. Results are shown in Figure 1A. RNA was completely degraded in formalin-incubated tissue after only 15 minutes indicated by the absence of the ribosomal bands. We repeated this protocol several times using different brands of formalin and obtained the same results (data not shown). Absolute ethanol did not degrade RNA after 15-minute, 1-hour, 4-hour, or 24-hour incubations. Xylene partially degraded RNA after 1 hour, but not after 15 minutes. The latter observation is important because conventional overnight tissue processing usually includes two 1-hour xylene cycles. To our surprise, however, tissue that was incubated in 0.9% sodium chloride yielded intact RNA with preserved ribosomal bands at all tested time points up to 24 hours.

We further pursued the saline studies because of traditional belief that for preservation of intact RNA, the tissue should either be snap-frozen or immediately used (Sambrook and Russell, 2001). Because in the initial experiments we used B. Brown (Irvine, California) 0.9% sodium chloride, we tested the possibility of it containing other chemicals that may protect the RNA. Therefore, we repeated the experiments with Baxter saline (Deerfield, Illinois) and with our own "homemade" 0.9% sodium chloride. We obtained the same results suggesting that it is the sodium chloride solution itself that protects RNA. We also extended the incubation of tissue in saline for up to 1 week, but in these experiments the quality of extracted RNA was inconsistent (data not shown). Also, to test whether increase in the temperature will eventually lead to RNA degradation, we incubated tissue for 15 minutes at temperatures ranging from 4° C to 65° C. We found (Fig. 2) that RNA was intact at 42° C, but it became partially degraded at 50° C and almost completely degraded at 55° C.

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Comments

Every molecular biology textbook or manual ascertains that the key to successful purification of high quality RNA from cells and tissue is speed. Therefore, to isolate intact RNA, prompt removal of tissue followed immediately by snap freezing in liquid nitrogen

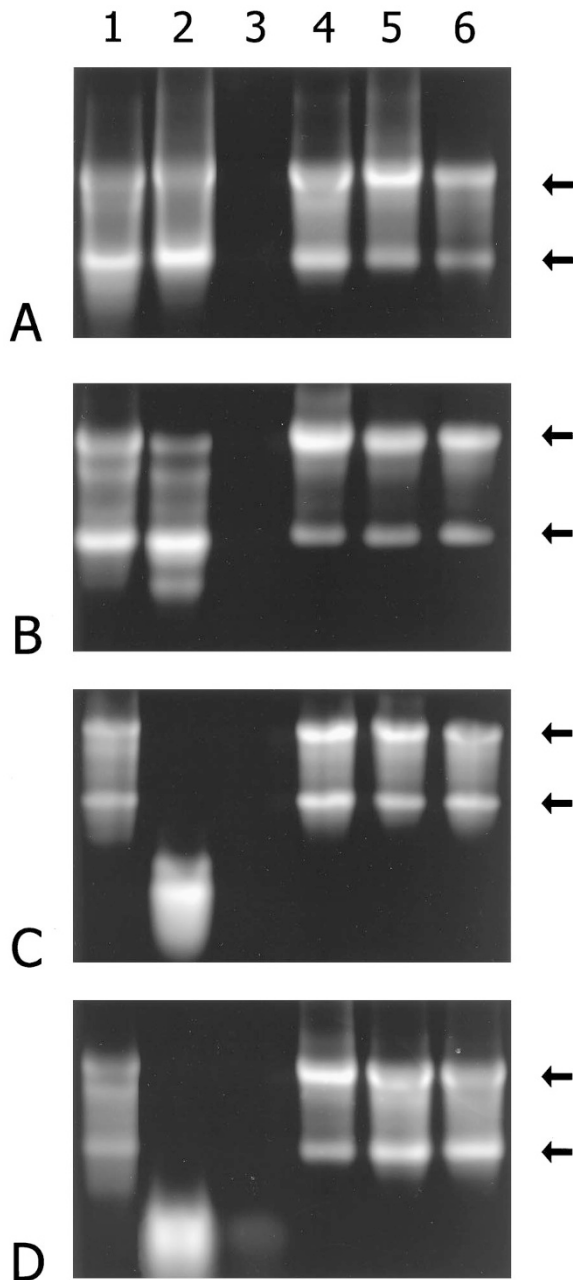


Figure 1. Total RNA extracted from mouse liver incubated at room temperature for 15 minutes (A), 1 hour (B), 4 hours (C), and 24 hours (D) with different chemicals (lane 1: 100% ethanol; lane 2: xylene; lane 3: 10% formalin; lane 4: RNA Later; lane 5: snap frozen tissue; lane 6: normal saline). Position of 28S and 18S ribosomal RNA is indicated by the arrows.

is required (Davis et al, 1986; Sambrook and Russell, 2001). Although it is routinely utilized by researchers, quick-freezing is inconvenient and impractical outside the laboratory environment. Similarly, placement of liquid nitrogen or dry ice containers in the operating rooms is often impossible or, even when feasible, is very inconvenient. Delays in transport and/or immersion in appropriate preservative solutions may explain why RNA in surgical specimens is often degraded by the time it reaches the laboratory. The fact that surgically removed samples can now be immersed and

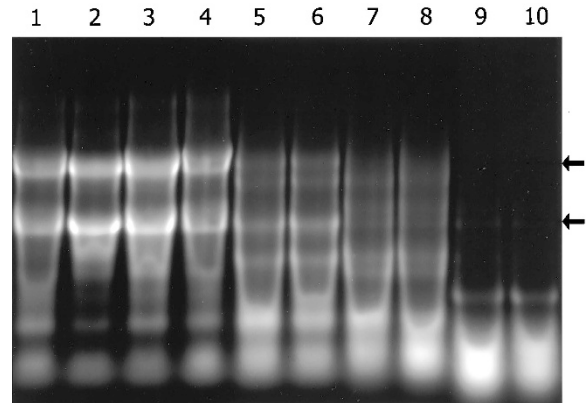


Figure 2. Total RNA extraction from mouse liver incubated with normal saline for 15 minutes at 37° C (lanes 1, 2), 42° C (lanes 3, 4), 50° C (lanes 5, 6), 55° C (lanes 7, 8), and 62° C (lanes 9, 10). Position of 28S and 18S ribosomal RNA is indicated by the arrows.

transported in an easily available and inexpensive saline solution—that protects RNA for at least 24 hours—opens new possibilities for molecular studies on human tissue.

It is not clear why isotonic saline solution preserves RNA, although we can speculate that normal saline protects the integrity of cell membranes and, hence, prevents the release of intracellular RNase. Other unknown mechanisms may also play a role in preventing RNA degradation in tissues preserved in saline.

In conclusion, because formalin, the most commonly used tissue fixative, is the most damaging to tissue RNA, it is logical to search for alternative fixatives/preservatives that combine good histomorphology with preservation of RNA. Until such a time, however, normal saline solution can serve as RNA preservative, eliminating the need for snap-freezing tissue. Saline could also serve as a short-term (24 hour) transport medium between the operating rooms or outpatient clinics and the laboratory.

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