# CURRENT METRICS OF THE QUALITY OF HUMAN PROSTATE TISSUE ARE INADEQUATE TO PREDICT QUALITY OF RNA AND PROTEIN

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## Abstract

**Background: Human tissue is subjected to pre-analytical conditions before preservation that impact tissue quality. Some conditions are known to affect gene expression levels, i.e. duration of warm ischemia, duration of autolysis, high protein pre-operative diet. Developing a metric to assess preservation of RNA, DNA, protein could help to both select out for experiments low quality samples and develop a tissue preservation fingerprint for normalizing gene expression levels.**
**Materials and methods: We assessed multiple metrics of tissue quality in snap frozen samples from 50 prostatectomies (time interval of vessel ligation to receipt in the lab, time interval to freezing, and Bioanalyzer RIN) and from 15 rapid autopsies (following death from metastatic prostate cancer) - histologic preservation, percent of necrosis, time interval between death and freezing, and Bioanalyzer RIN. We also evaluated immunohistochemically defined expression of beta-actin and beta-2-microglobulin by 20 primary prostate cancers and of PSA and PSAP in 8 prostates where tissue aliquots were let autolyze in a humid atmosphere at either 37C or 5C for time intervals of between 1 and 56 hours.**
**Results: RIN had virtually no correlation with time to freezing of prostatectomy tissue (range: 20 to 70 minutes) or of rapid autopsy samples (range: 1.5 to 6 hours; r < 0.9). Extent of necrosis of autopsy samples (range: 0 to 20%) did not predict RIN. Stain intensity of immunoreactive PSA and PSAP was variable, decreasing by 3 hours in tissue kept at 37C and by 4 hours in tissue kept at 5C. However, some samples kept at 5C for 32 were indistinguishable from 1 hour samples. Finally, immunoreactivity for beta-actin and beta-2-microglobulin was highly heterogeneous within a sample.**
**Conclusion: Better metrics of tissue quality, of nucleic acid and protein integrity, and reference housekeeping genes are urgently needed.**
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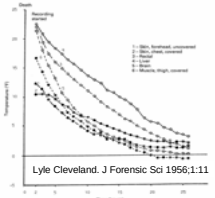
## Background

Preservation of and assessment of preservation of macromolecules (DNA, RNA, protein) in human tissue is critical to obtain and use high-quality tissue specimens for translational research. The following metrics have been used to assess integrity:

- Histology (a global measure of tissue quality)
- Retention of immunoreactivity (an assay of proteins)
- Retention of intact RNA (assessed here by Bioanalyzer)

Although these parameters and surrogates assays of tissue preservation, such as time and temperature of tissue, can be used to try to predict the integrity of macromolecules, none of these appear to be accurate for individual tissue samples.

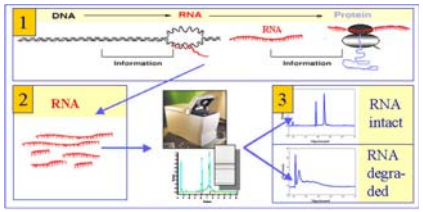

Lyle Cleveland. J Forensic Sci 1956;1:11

Recent studies have identified RNA expression profiles for high caloric pre-op diet and warm and cold ischemia. In this poster we report our studies of tissue preservation.

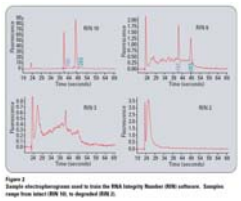
## Methods

We studied unfixed tissue samples from our prostate cancer research program,. These consist of 1,800 radical prostatectomy samples, pre- prostatectomy needle biopsies, and samples from our rapid autopsy program, which has collected fresh tissue from > 90 men who died of progressive prostate cancer.

Values that we recorded included time intervals in tissue handling, histologic preservation, percent of necrosis, immunohistochemically defined reactivity (intense, moderate, faint, focal, none) and Bioanalyzer RIN values (measurements of RNA integrity)




Schematic of Bioanalyzer analysis of RNA integrity. RIN numbers (range 2 to 10 illustrated to the right) are based on the distribution of sizes of RNA.



## Results

### Histology

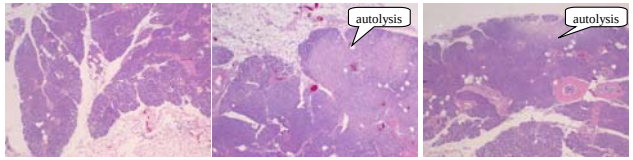



Figure. Three separate regions from an autopsy pancreas (standard hematoxylin and eosin stains).. Note the heterogeneity in size and distribution of foci of autolysis, which have no apparent correlation with body temperature, tissue temperature, or location in the pancreas. Histology is a coarse metric for tissue preservation.

### Immunohistochemistry

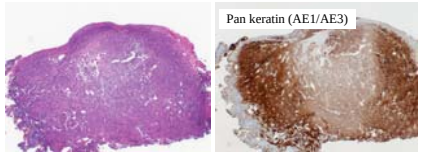



Figure. A carcinoma, which replaces most of a lymph node, expresses keratin (based on an anti-pan-keratin stain). This is an extreme example of heterogeneity of immunoreactivity due to artifact. The center of the tumor focus (ctr) should exhibit the same intensity of pan-keratin staining as the edge (edge) of the tumor focus.

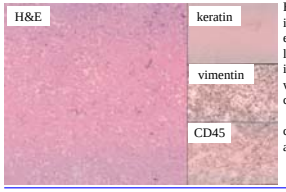
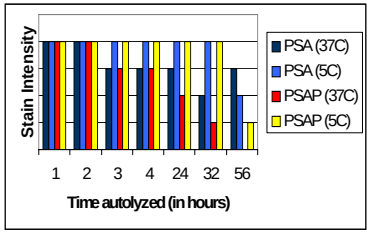


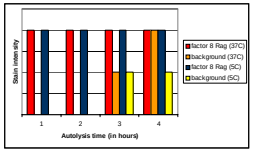

Figure. A lymphoma, which is totally necrotic, cannot be identified as a population of cells based on hematoxylin and eosin stains (H&E). Although the necrotic tumor cells have lost their histologic structure, they have retained their immunophenotype (vimentin to a greater degree than CD45, which has lost most of its membrane pattern.. Lymphomas characteristically lack keratin and express vimentin and CD45.

For a totally necrotic tumor that has no residual histological cell integrity to retain intact immunoreactivity is not rare, although it is:

- not predictable based on any tissue handling parameters
- and, retention of immunoreactivity varies by antigen

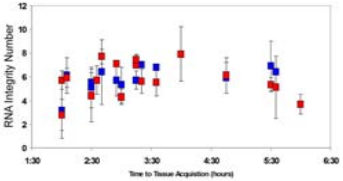



Prostates let autolyze for up to 56 hours at 5 and 37 C, then stained with anti-PSA and anti-PSAP. Note loss of immunoreactivity, differing by time, temperature of autolysis, and antigen.

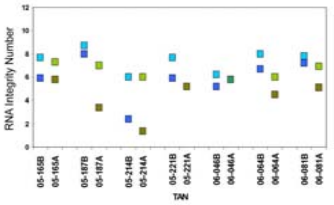



Anti-Factor VIII Related antigen (vWF) stain of prostate cancer. Although specific staining is equally intense over 4 hours at 5 and 37C, background stain increase after 2 hours, more rapidly in the 37C specimens.

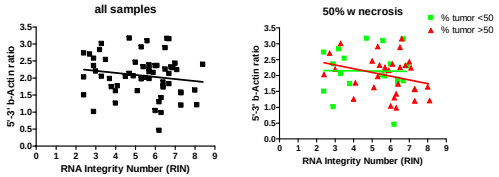
### RNA integrity




This graph shows the RIN numbers for rapid autopsy tissues from time of death to time tissue was frozen. Normal tissue is blue (n>3/patient), tumor tissue is red (n>3/patient). The maximum RIN value is 10 (= highest quality with optimal preservation of RNA (intact RNA)).




This graph shows the RIN numbers for paired (by rapid autopsy) muscle (B-blue) and liver (A-green) samples taken at the start of the autopsy (light hue) and at the end of the autopsy (dark hue). Note RNA degradation (decreased RIN value) for each set of paired samples.




Correlation between RIN number and ratio of 5' vs. 3' amplified housekeeping gene beta-actin, using RNA extracted from human prostate tissue samples. The ratio of 5' vs. 3' RNA is a potential indicator of quality of RNA in tissue samples. Note the poor correlation between RIN and 5' vs. 3' beta-actin (left). Note also the poor correlation between RIN and the extent of necrosis (right).

## Discussion

Based on our studies, we think that (1) better metrics for quality of tissue macromolecules need to be developed, and (2) identification of the steps in tissue handling that most affect quality of macromolecules need to be undertaken. These steps span the range from host-pre-procedure status (diet, duration of anesthesia, relevant SNPs) to assay (level of specific mRNAs and per cell protein expression level).