



Measuring Biomarker Stability in Frozen Tissue

Post-acquisition variables:

- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- **Rate of freezing**
- Freeze / Thaw
- **Size of aliquots**
- **Storage temperature**
- **Storage duration**
- **Storage in vacuum**
- **Processing methods**

NCI Best Practices for Biospecimen Resources

- Technical Operations Best Practices
 - **Collection, processing, storage**, retrieval, use
 - Collection and managing clinical data
 - **QA/QC**
 - Biosafety
 - Informatics, inventory control and tracking
- Ethical, Legal and Policy
 - Custodianship
 - Informed consent
 - Privacy protection
 - Access to biospecimens and data
 - Intellectual property and resource sharing

Principals of Evidence Based Biospecimen Research

- What practices affect the quality of a specimen?
- Can they be measured?
- Do the measurements have meaning to data acquired in research testing?

Frozen Tissue

- Still the pristine specimen carrying intact non-chemically altered DNA, RNA, and proteins.
- Will be needed for biomarker discover.
- May be needed for future clinical information that can't be resolved by robust assays.

Sample Packaging and Storage

- Can the quality of frozen specimens be protected with more stringent packaging?
- What is the duration of protection?

Questions

- Oxidation - RNA, DNA, & proteins
- Dehydration
- Morphological deformation
- Residual and active enzyme activity
- Storage container, temperature, length of storage

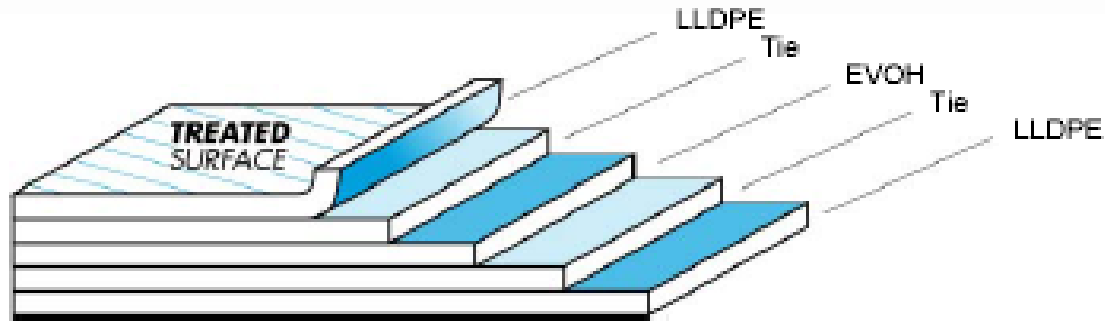


Operational Variables

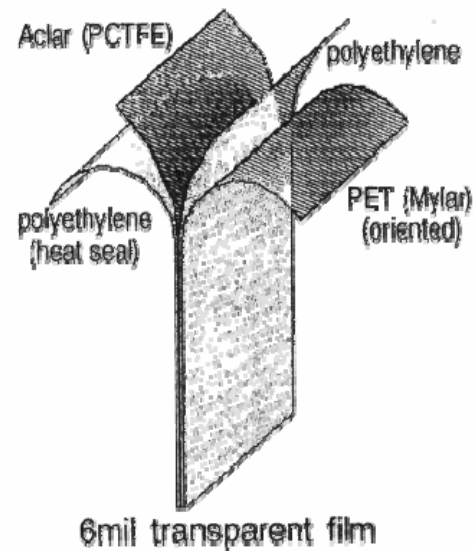
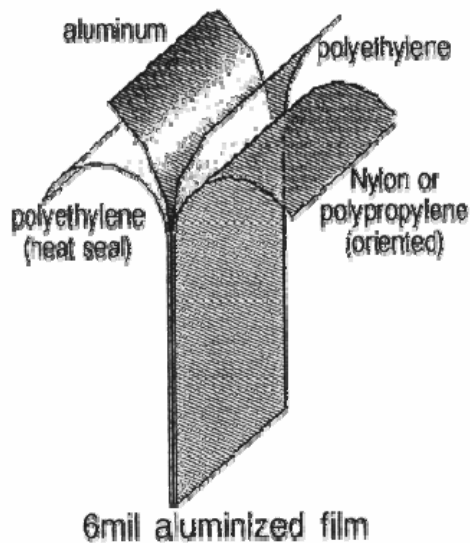
- Sample size
- Freezing rates
- Packaging analysis
 - Sealer
 - Pouch
- Tissue container



Polymer Laminate Diagrams



Typical Vapor Barrier Laminations



Proposed Solution



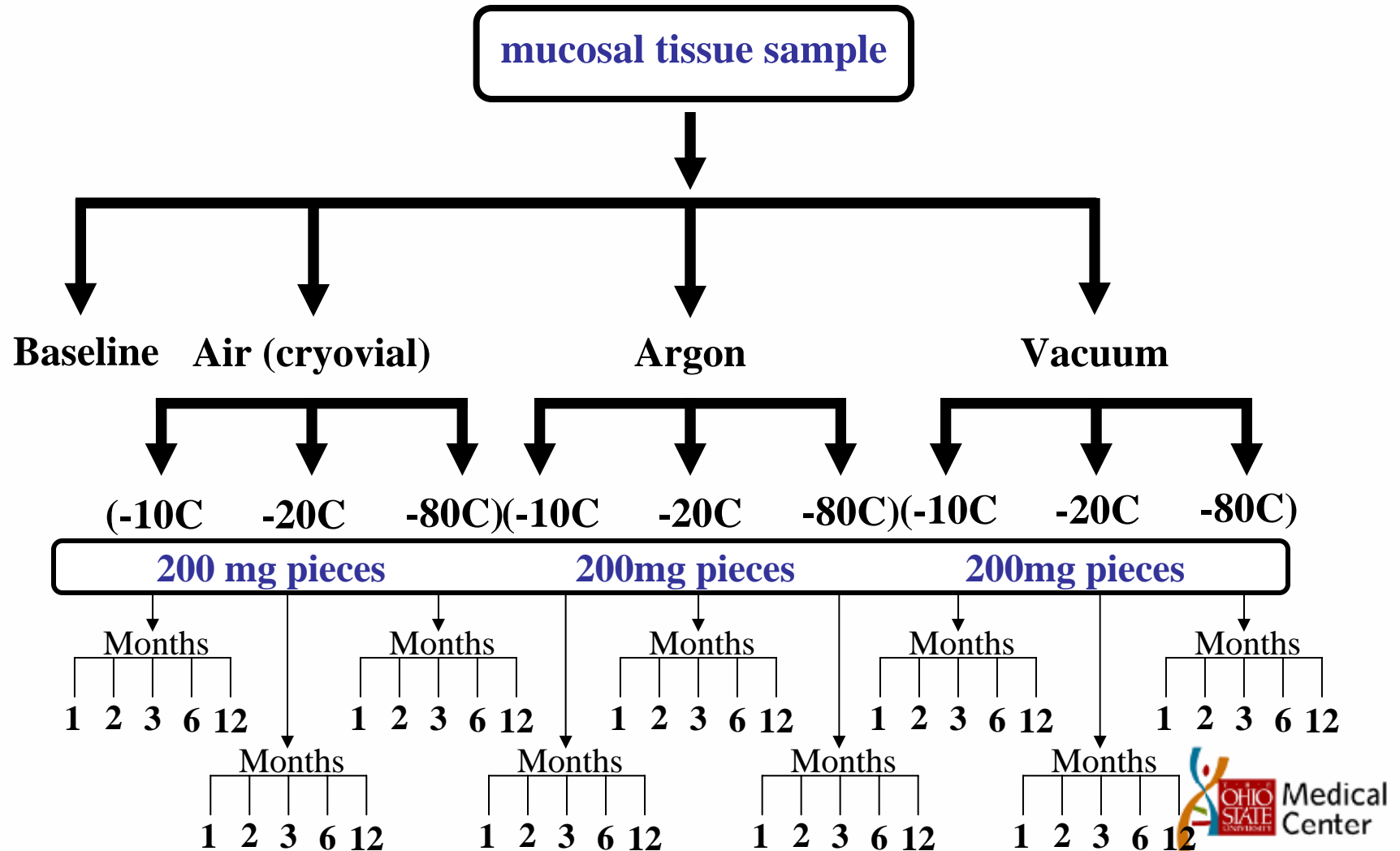
1. Foil laminate (high barrier packaging)
2. Embedding cassettes: protect tissue from distortion
3. Modified atmosphere packaging: vacuum or inert gas, i.e., argon

Vessel Freezing Periods

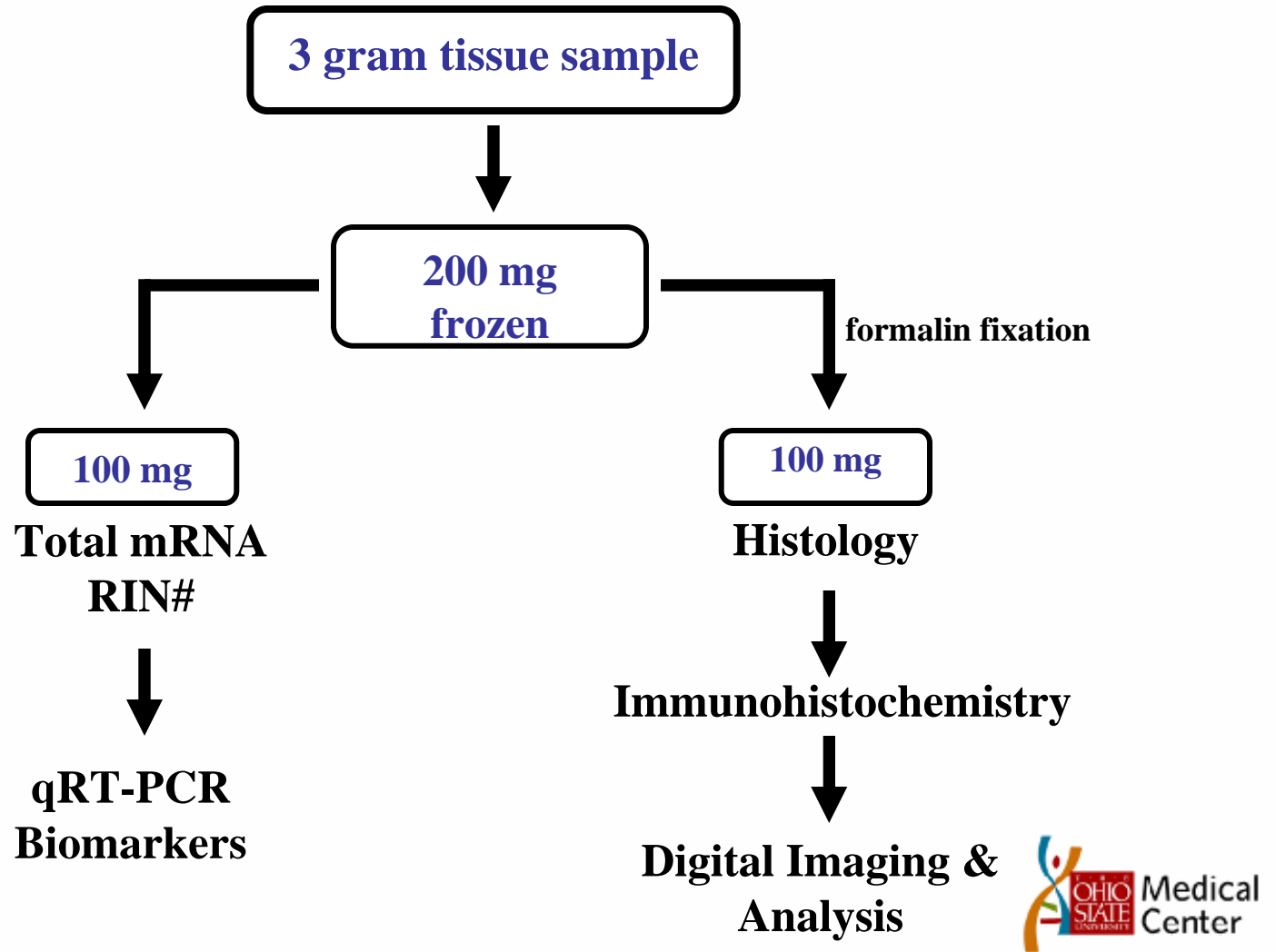
Based on the exhaustion of LN2 boiling	
Container	Time to Freeze (sec)
CRE tray in standard bag	50
Standard 5 ml tube	70
20 mil clamshell	23
30 mil clamshell	25
30 mil clamshell tray in bag (vacuum)	30
Barrier bag (vacuum)	26
Barrier bag (no vacuum)	30
Embedding tray in bag (vacuum)	30



Sample Dissection for a Single Case



Sample Dissection



Biomarkers

GAPDH - Glyceraldehyde 3-phosphate dehydrogenase (message)

- implicated in non-metabolic processes, transcription activation, initiation of apoptosis, and ER to Golgi vesicle shuttling – house keeping gene

CEA – glycoprotein-cell adhesion (protein & message)

- CEA is a tumor marker to identify recurrences
- In humans, the carcinoembryonic antigen family consists of 29 genes, 18 of which are normally expressed.

CDX2 (protein and message)

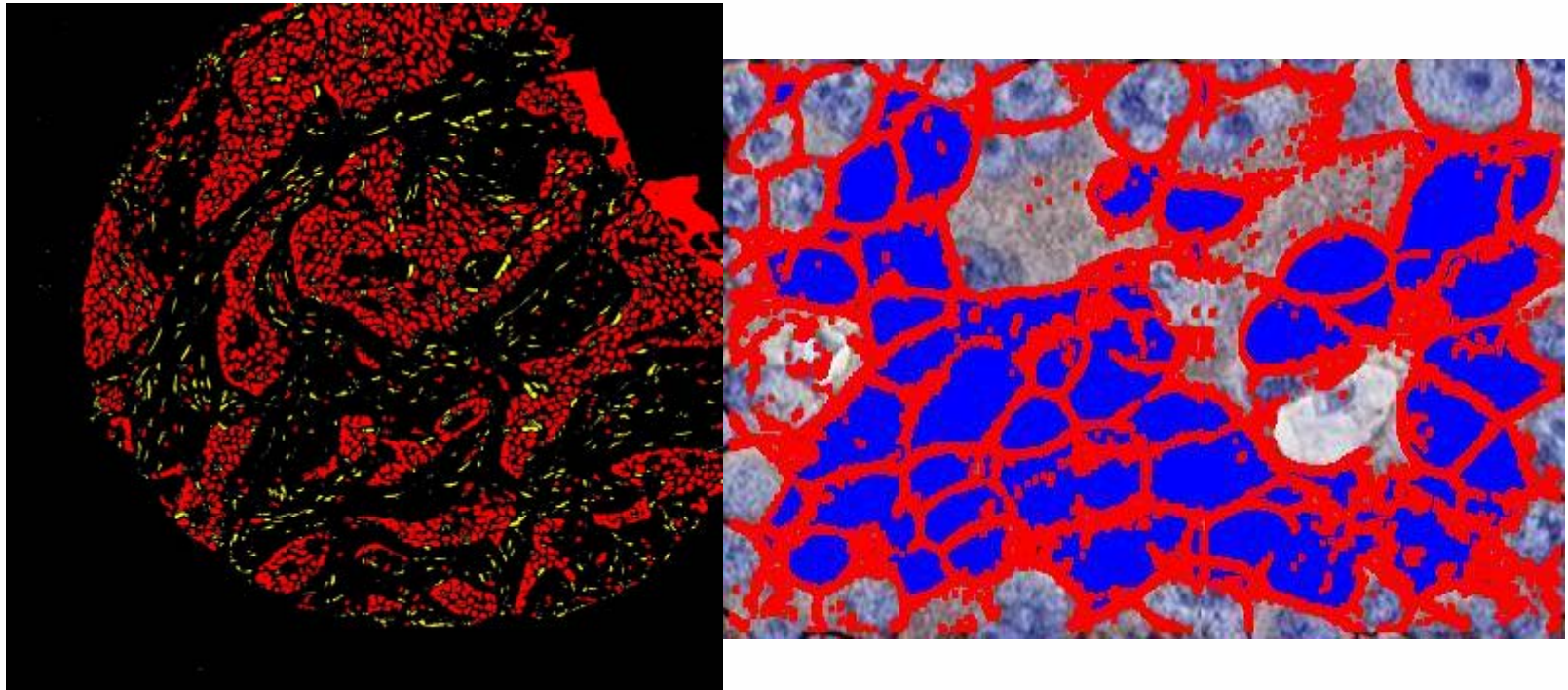
- CDX2 expression is maintained in the adult small and large intestinal epithelia
- CDX2 expression is induced or up-regulated in pathologic states

CK20 (protein)

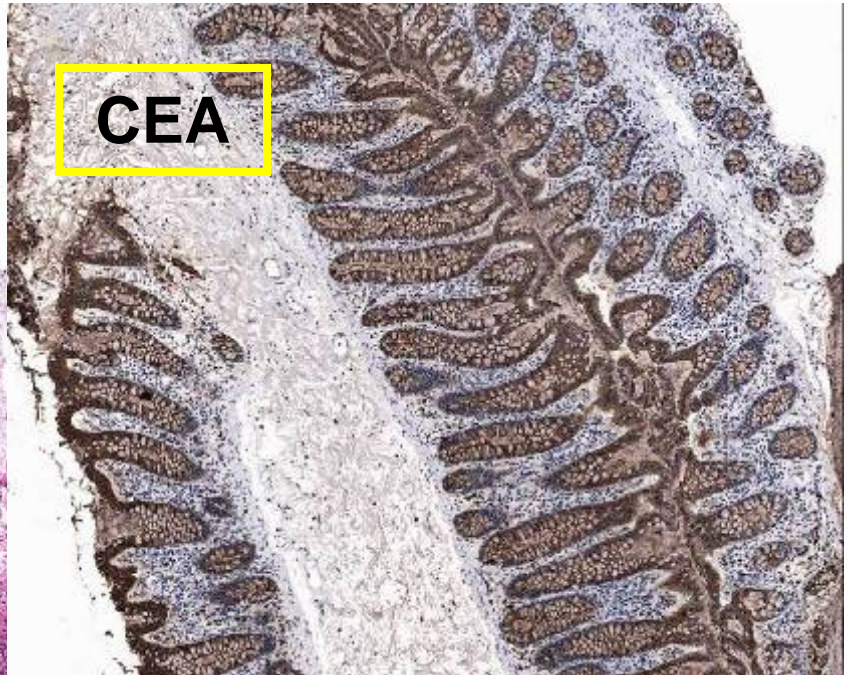
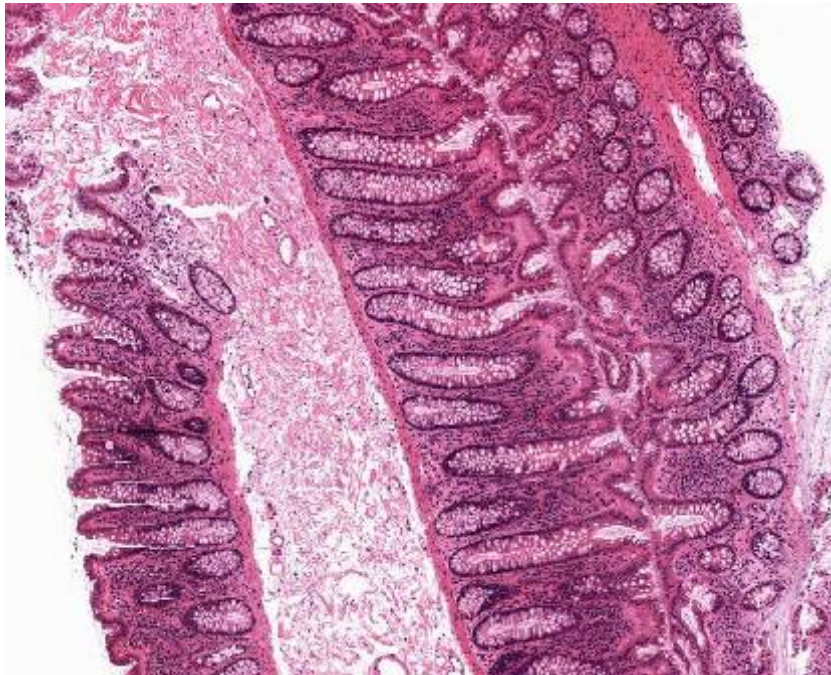
- cytokeratin, major cellular protein of mature enterocytes and goblet cells and is specifically expressed in the gastric and intestinal mucosa.

Elongation ratio

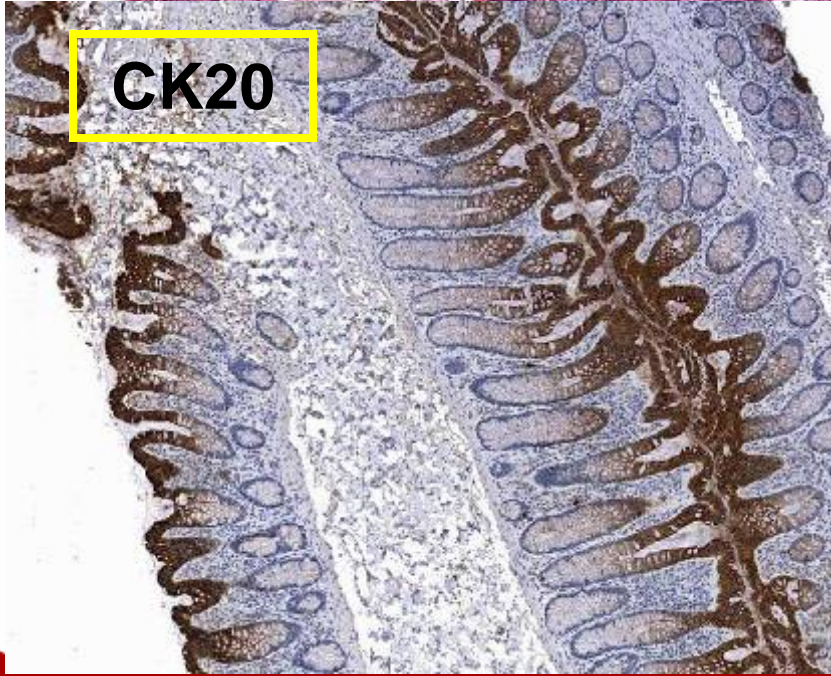
(Epithelial cells: red; Stromal cells: yellow)



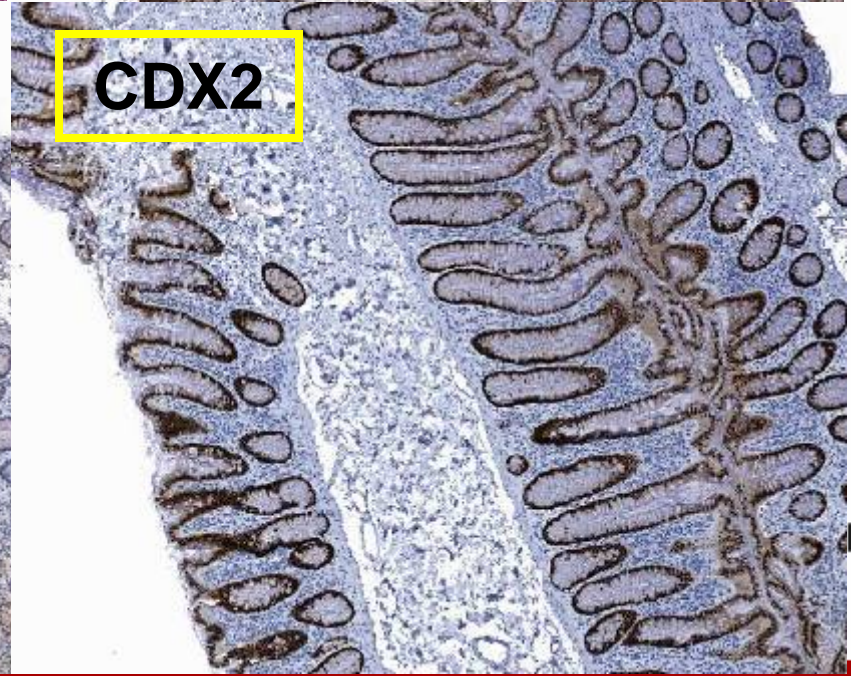
Courtesy of Sanford Barsky, M.D., Ph.D.



CEA



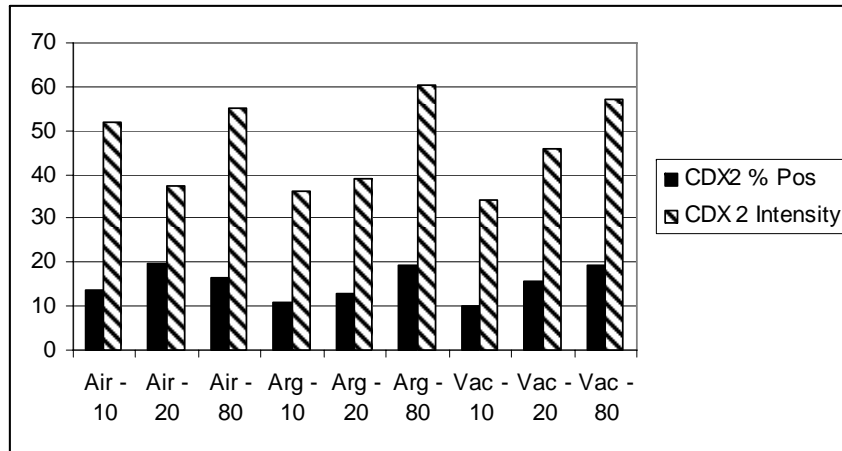
CK20



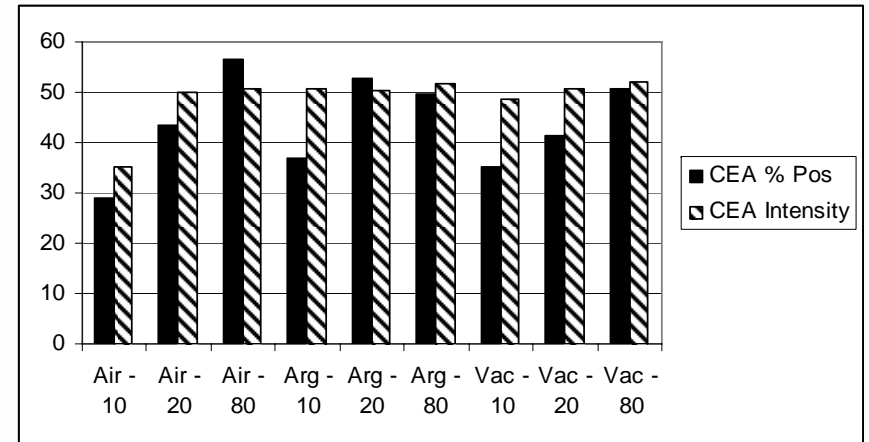
CDX2

Degree of change between different packaging methods versus storage temperature for CDX2, CEA, and CK20

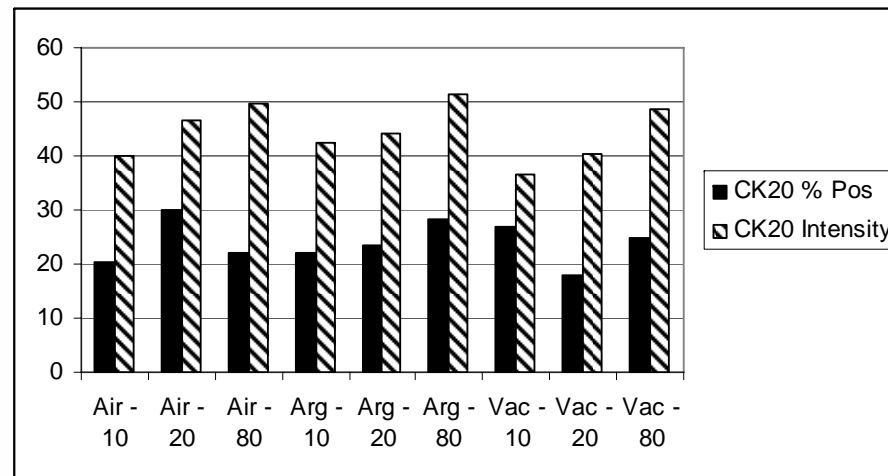
CDX2



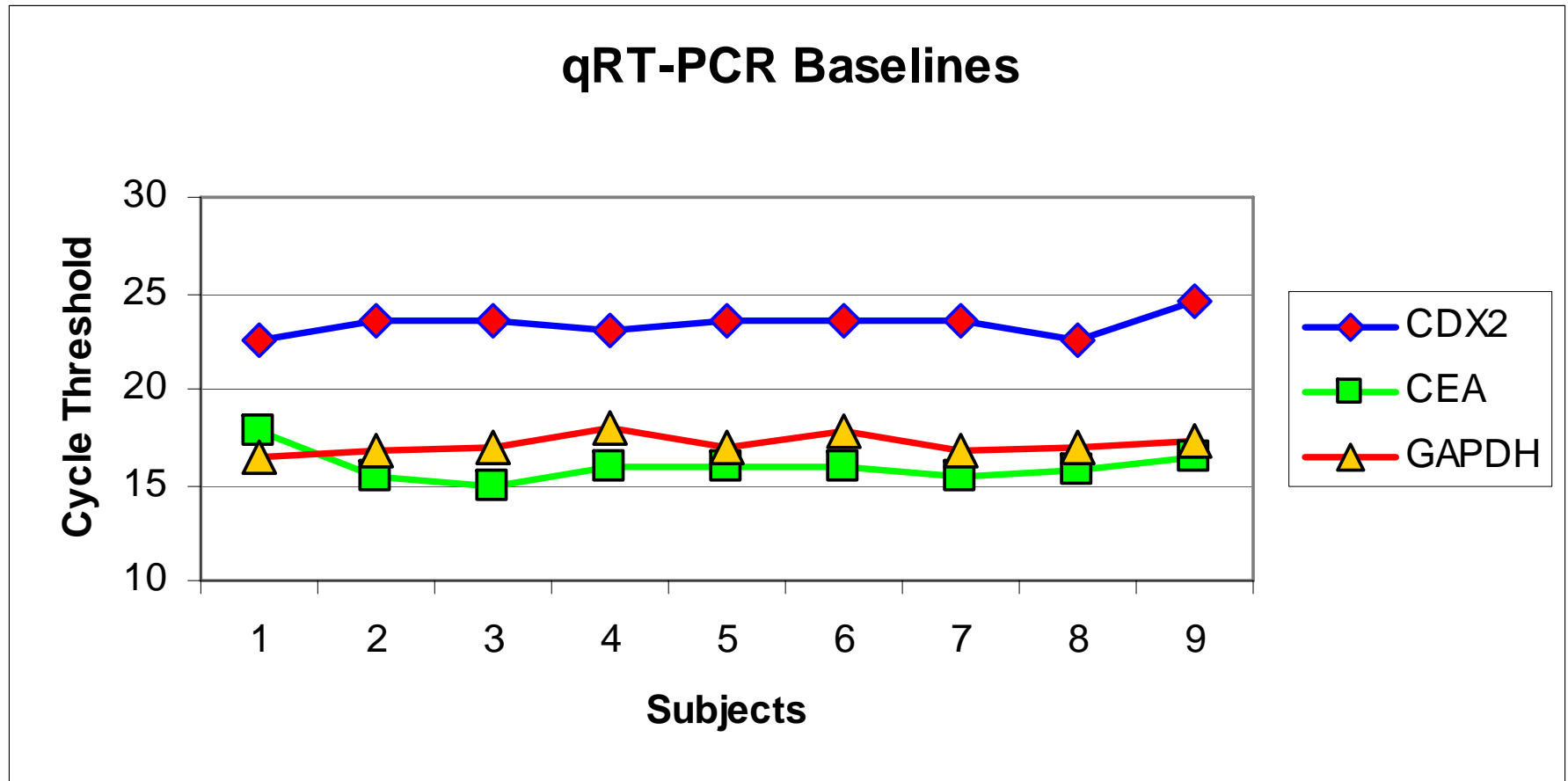
CEA



CK20

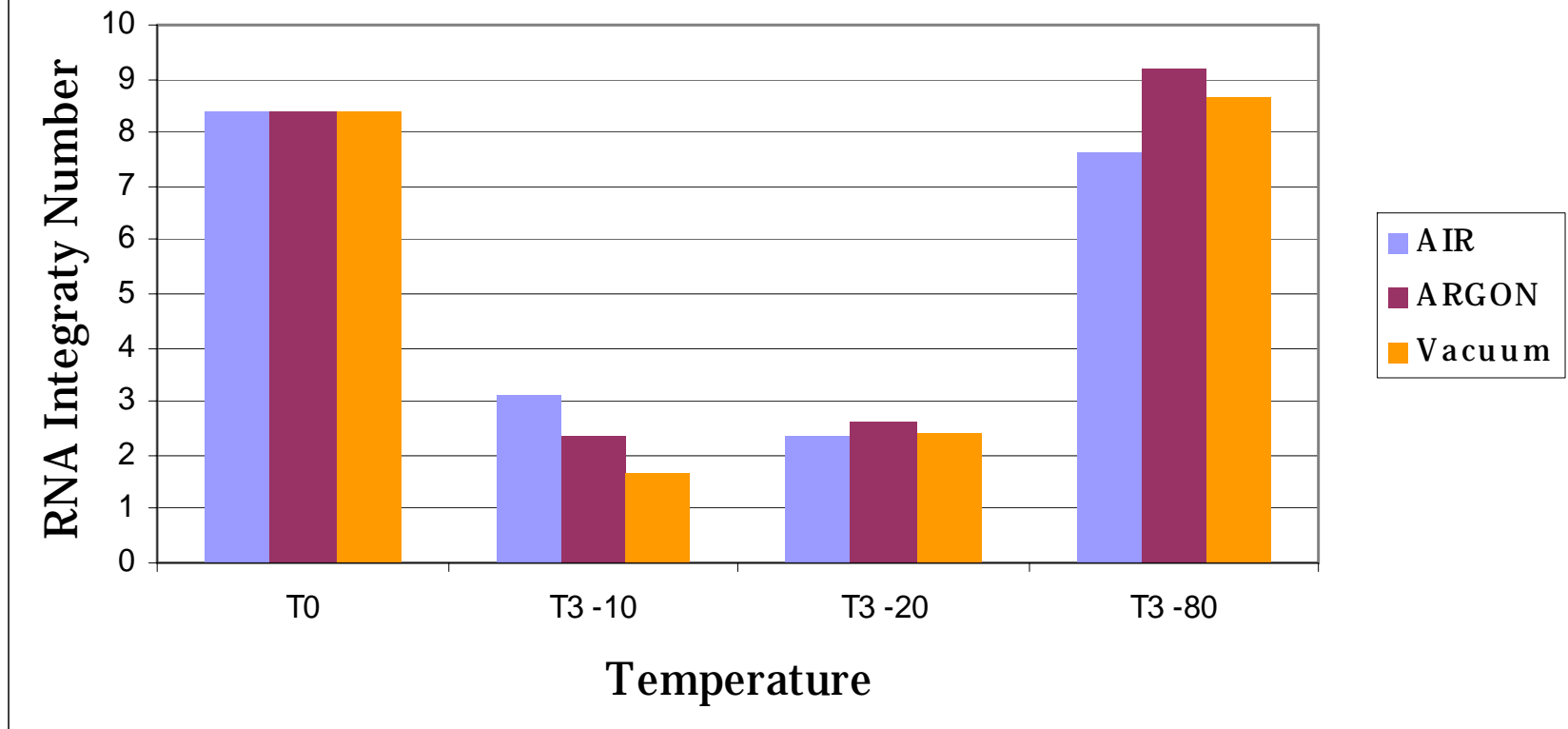


Measurement of Change in mRNA

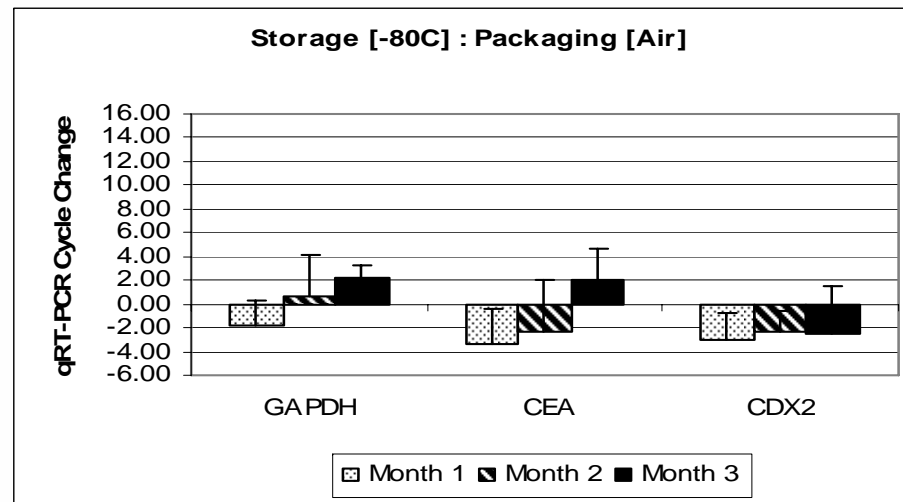
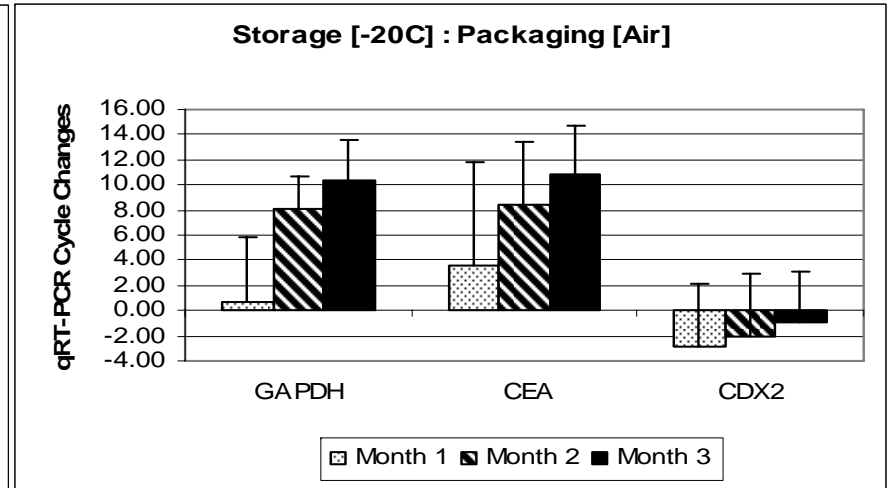
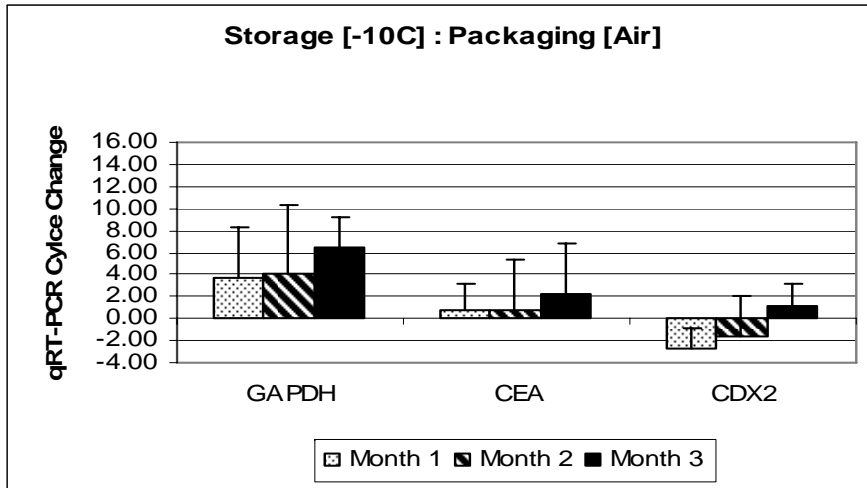


Cycles / Time point of storage - Baseline # cycles

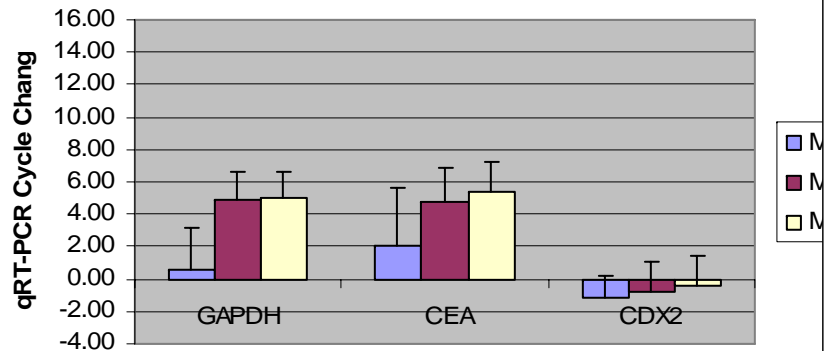
RIN Degradation Varying Temperature at Month 3



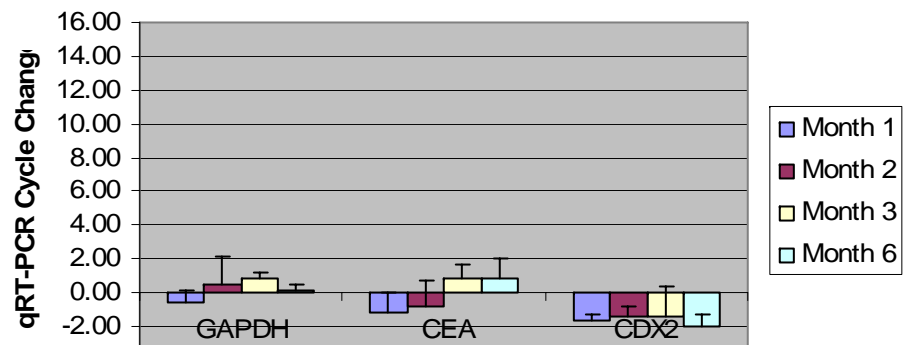
Changes in Biomarker qRT-PCR



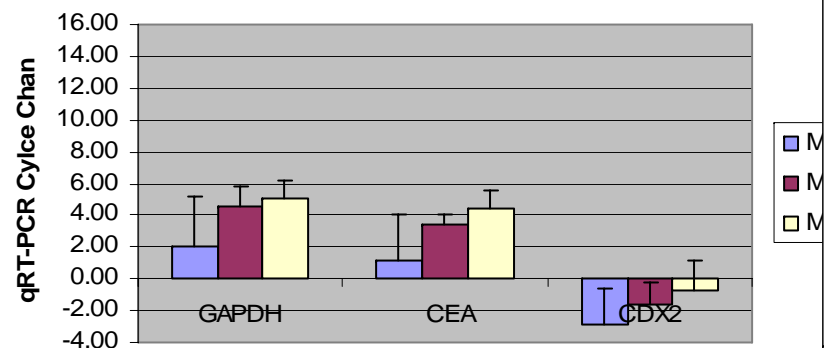
Storage [-20C] : Packaging [Air]



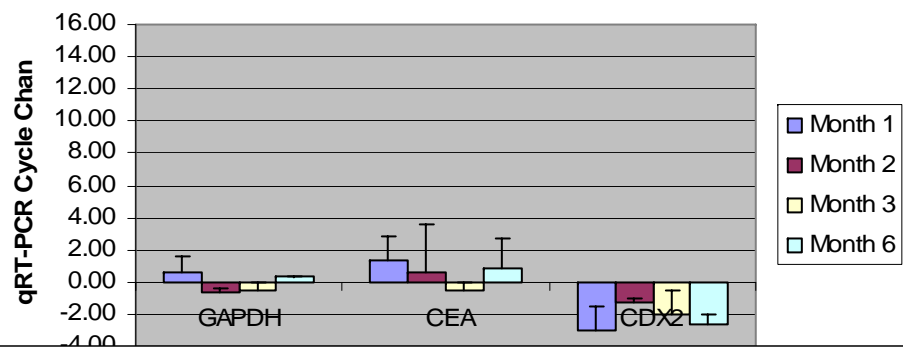
Storage [-80] : Packaging [Air]



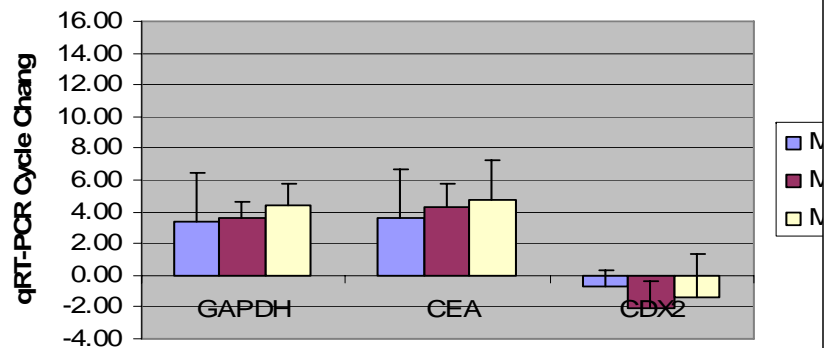
Storage [-20C] : Packaging [Argon]



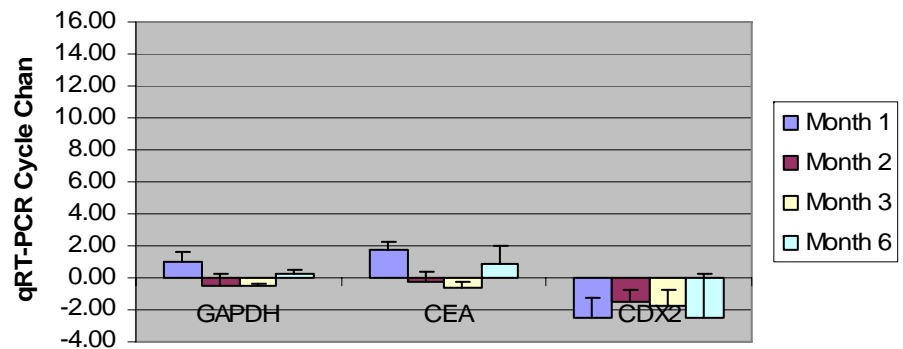
Storage [-80C] : Packaging [Argon]



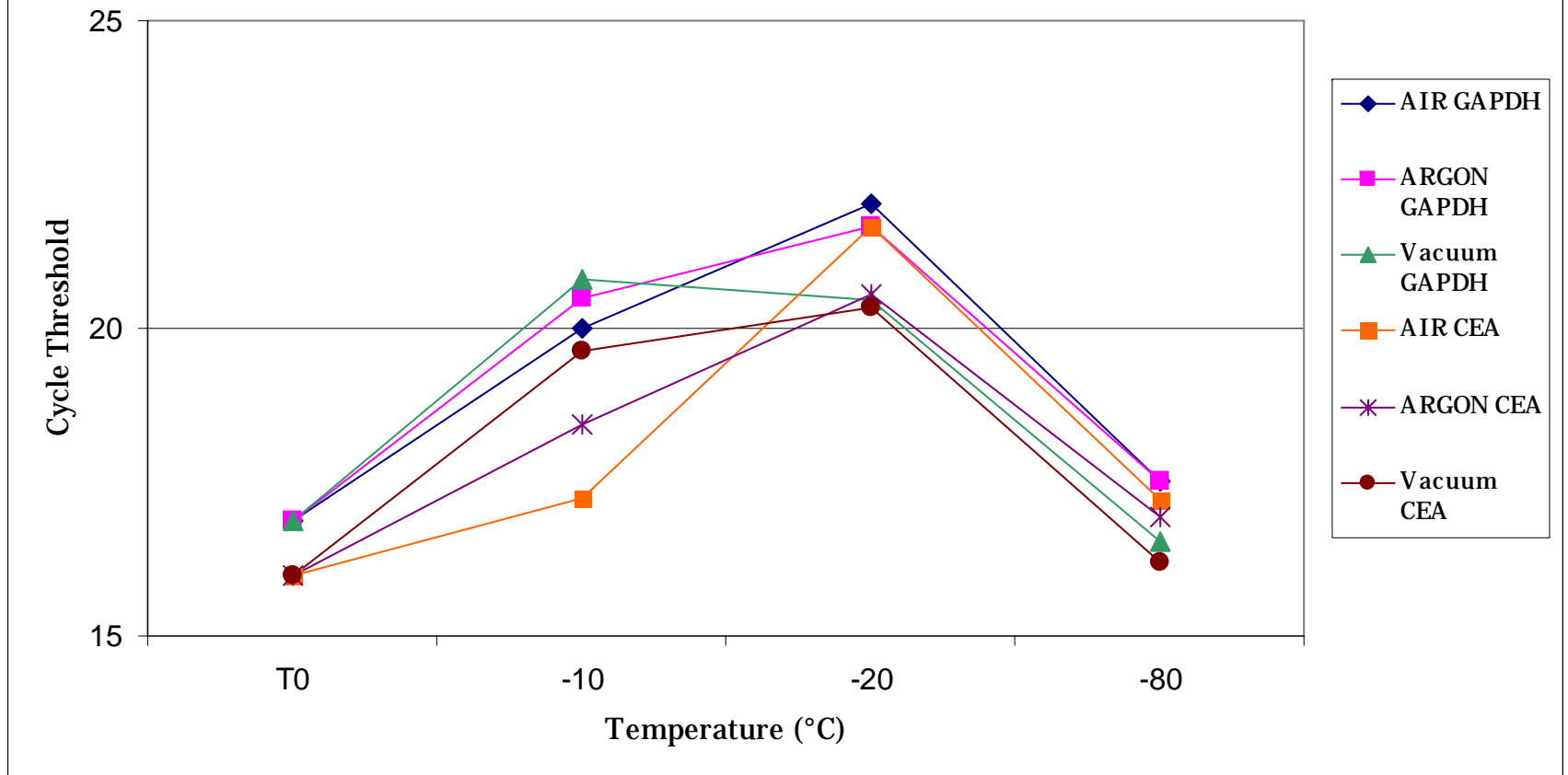
Storage [-20C] : Packaging [Vacuum]

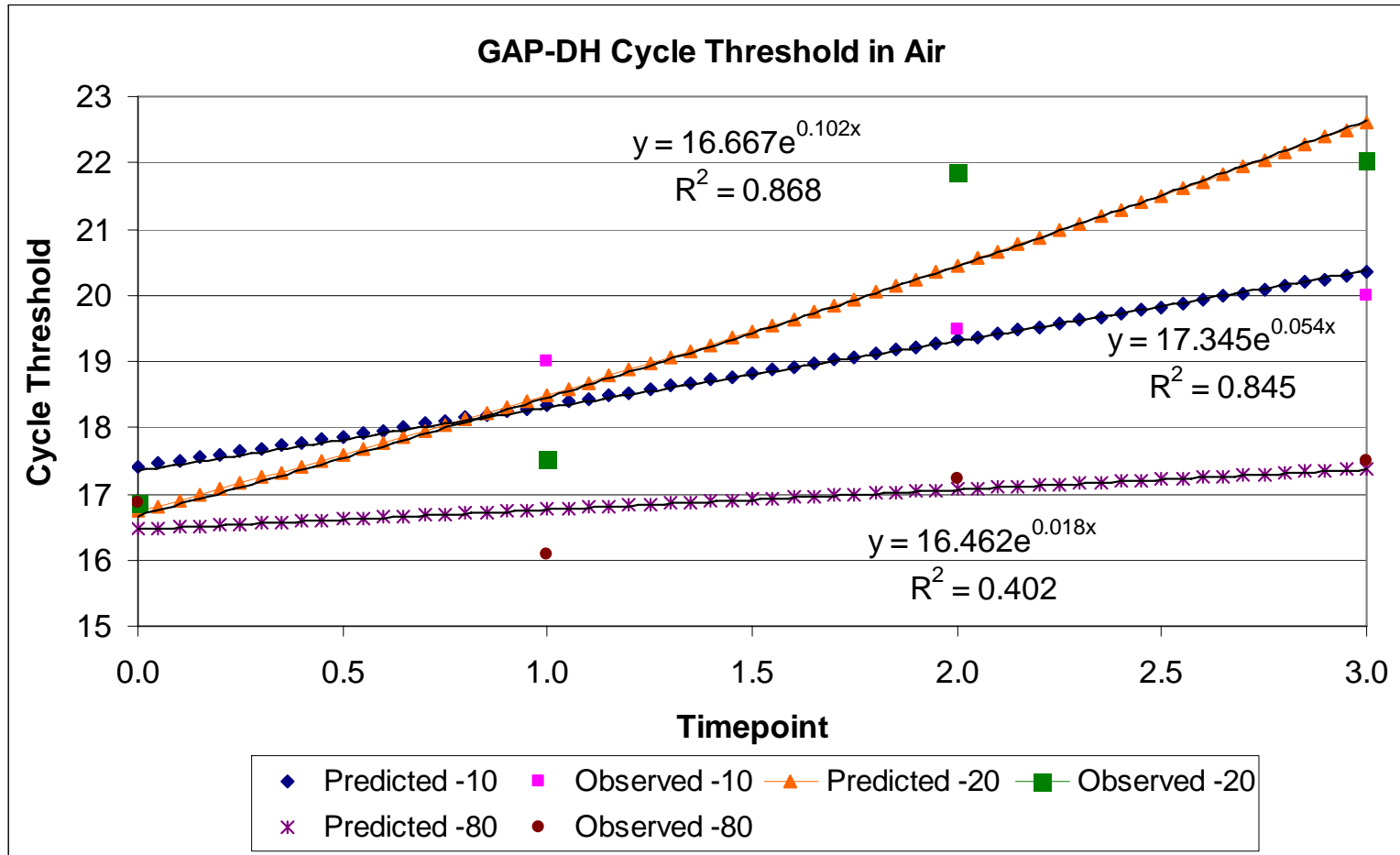


Storage [-80C] : Packaging [Vacuum]

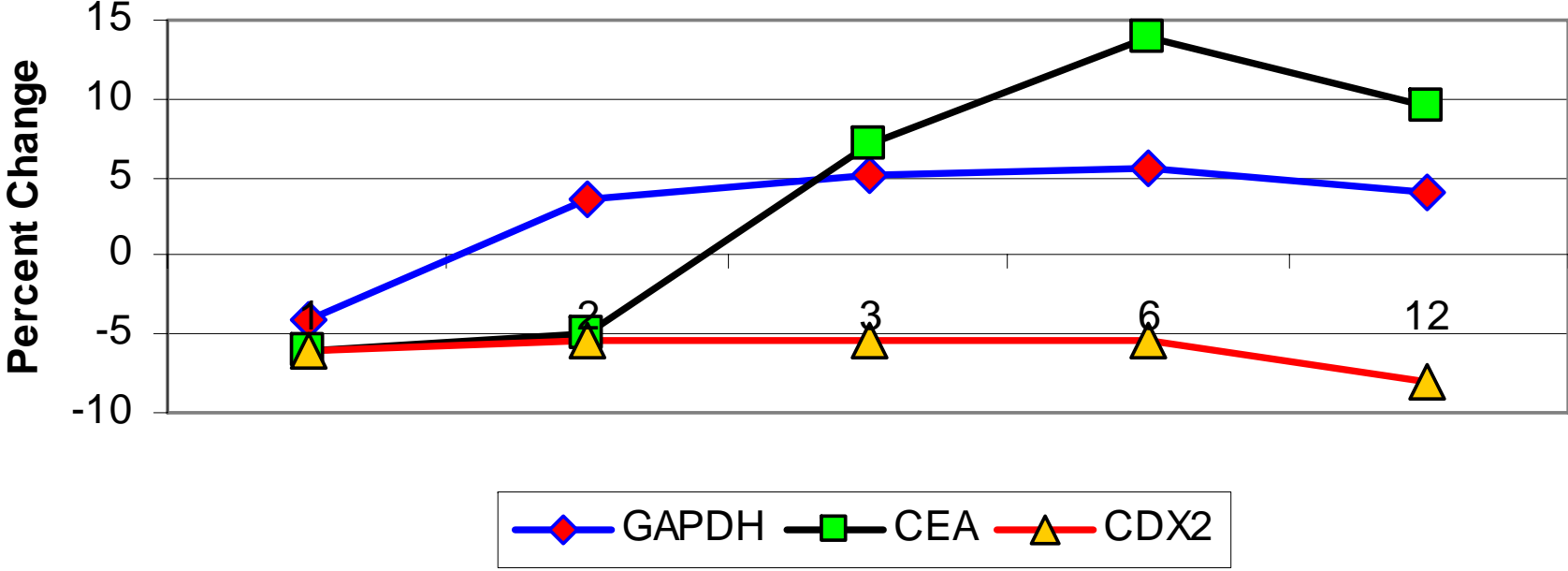


Biomarker Measurement Varying Temperature at Month Three

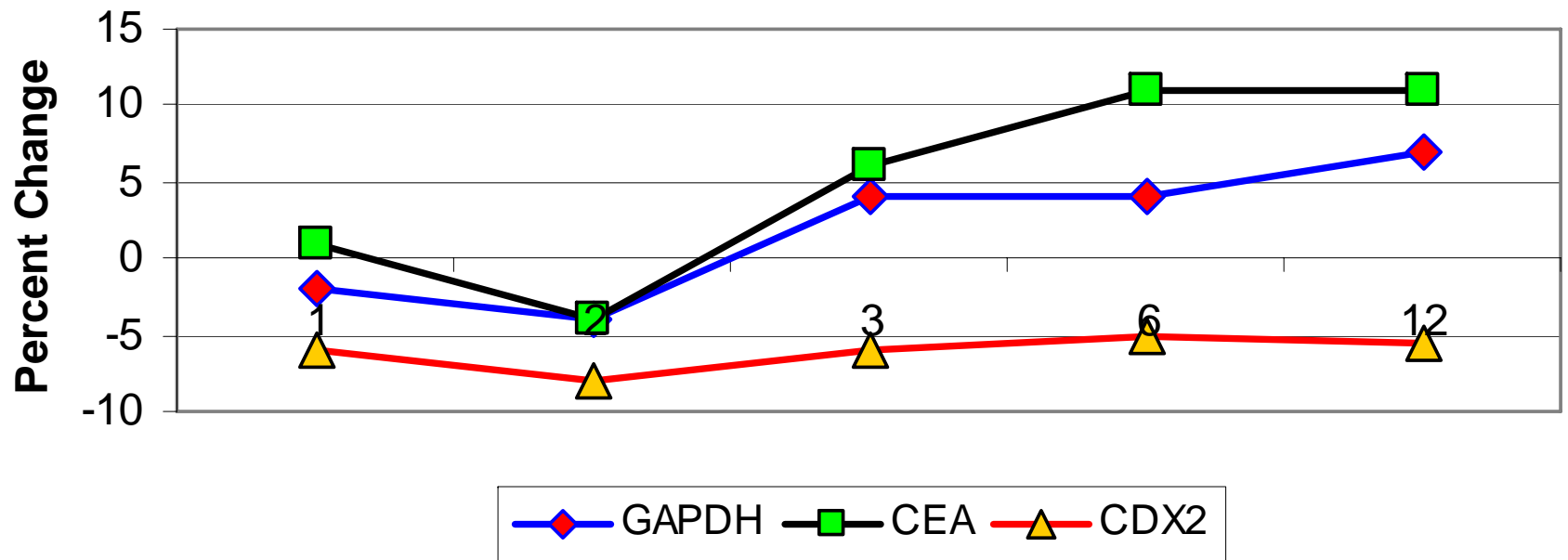




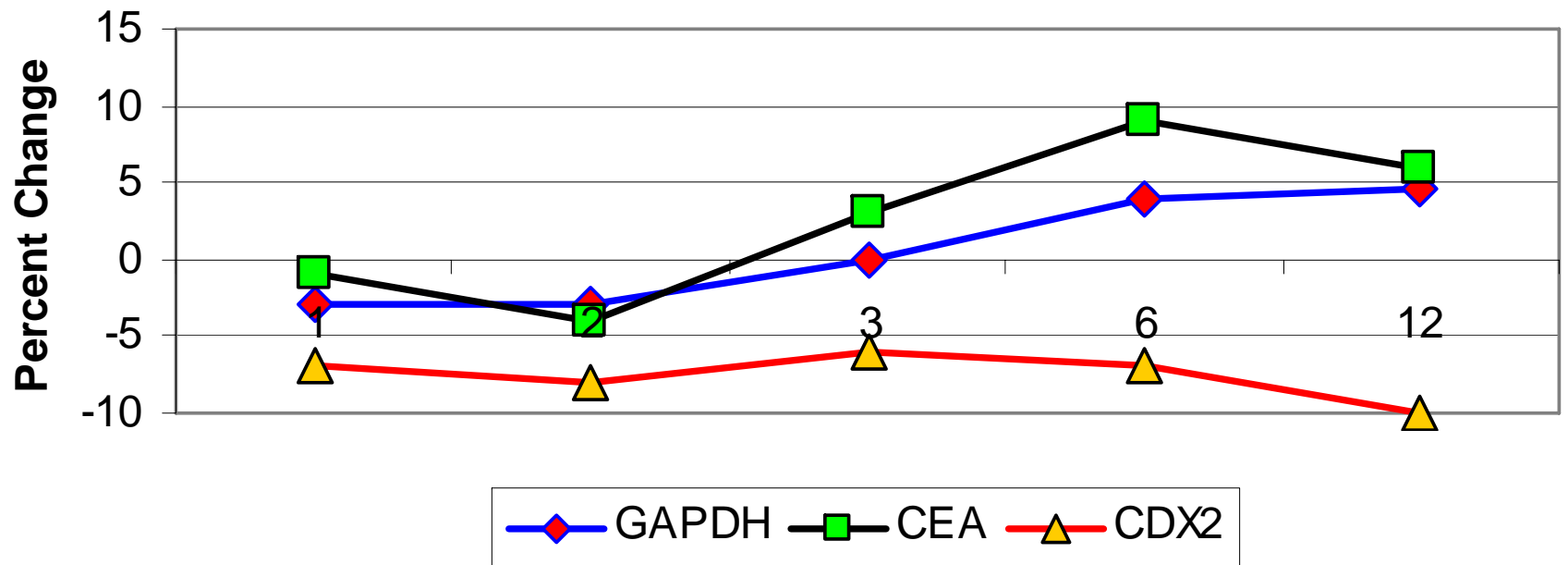
Air (cryovial) Packaging (-80C)

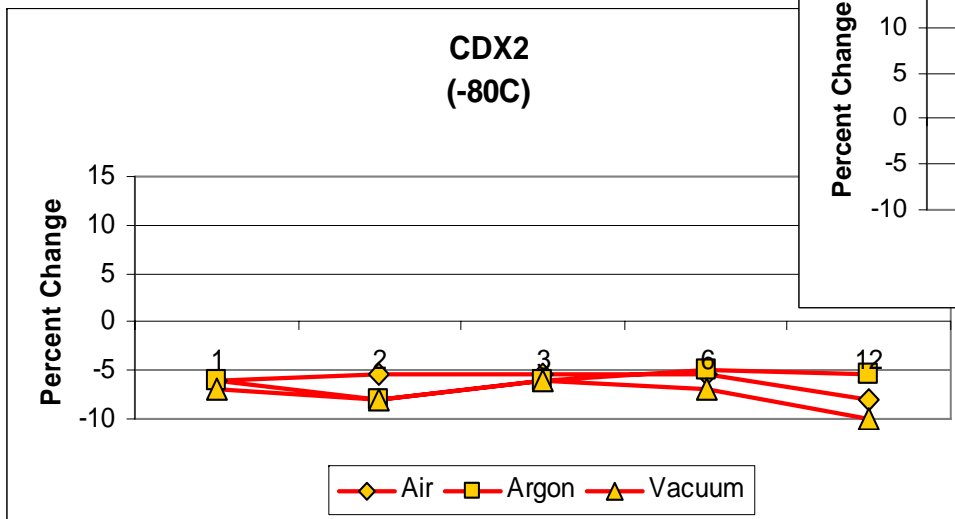
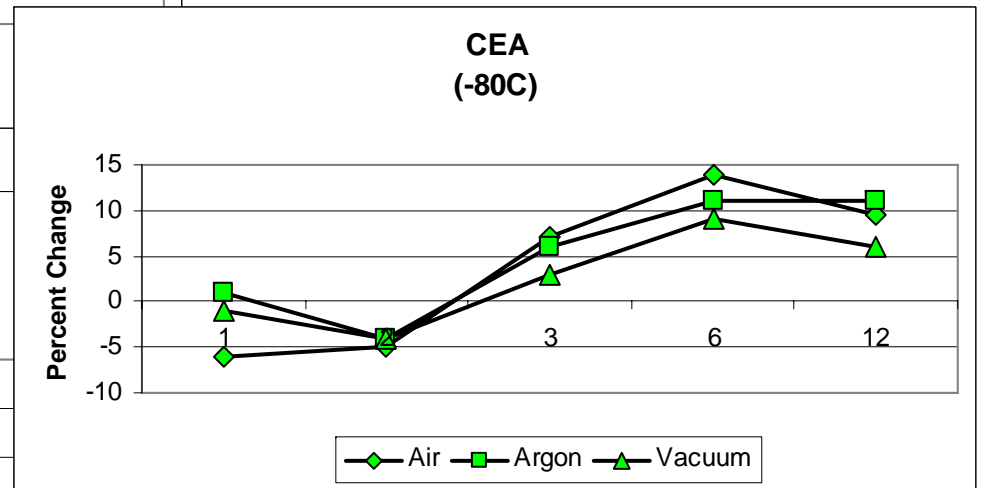
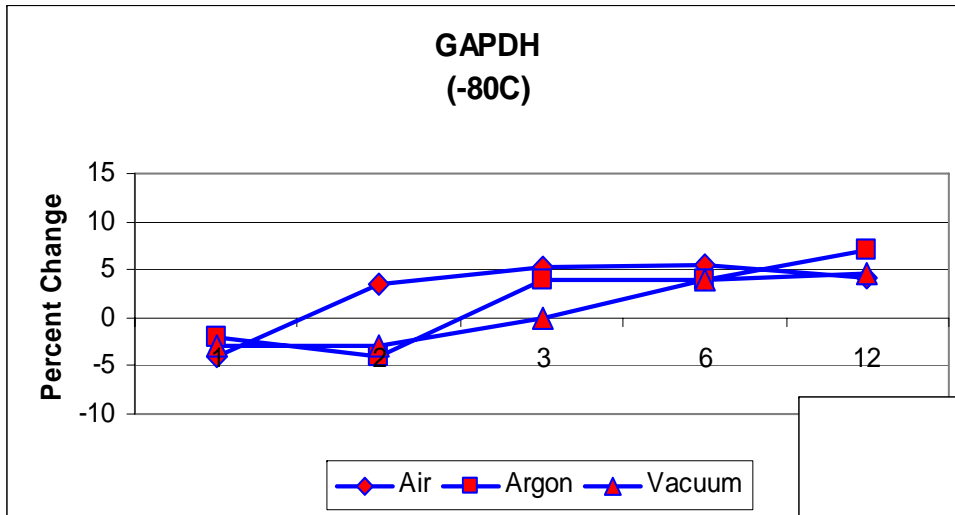


Argon Packaging (-80C)



Vacuum Packaging (-80C)





Conclusions / Future Studies

- Degradation occurs at about 5% in the first 12 months according to percent change in the original quantity of message
- No difference in packaging seen at this time
- Continue to measure the duration of time and packaging for affects in tissue quality
- Varying types of biomarkers need to be tested
- Compare fixation methods to the frozen tissue

Acknowledgments

Department of Pathology

- Dr. Yufang Tang
- Dr. Sanford Barsky
- Dr. Kurtis Yearsley
- Dan Rohrer
- Susie Jones
- Mary Marin
- Susan Long
- Nehad Mohamed

Department of Agriculture Engineering

- Dr. Gönül Kaletunç
- Jason Baker
- Wes Halderman*
- Sara Kallio
- Lily Meyer



Thank You!