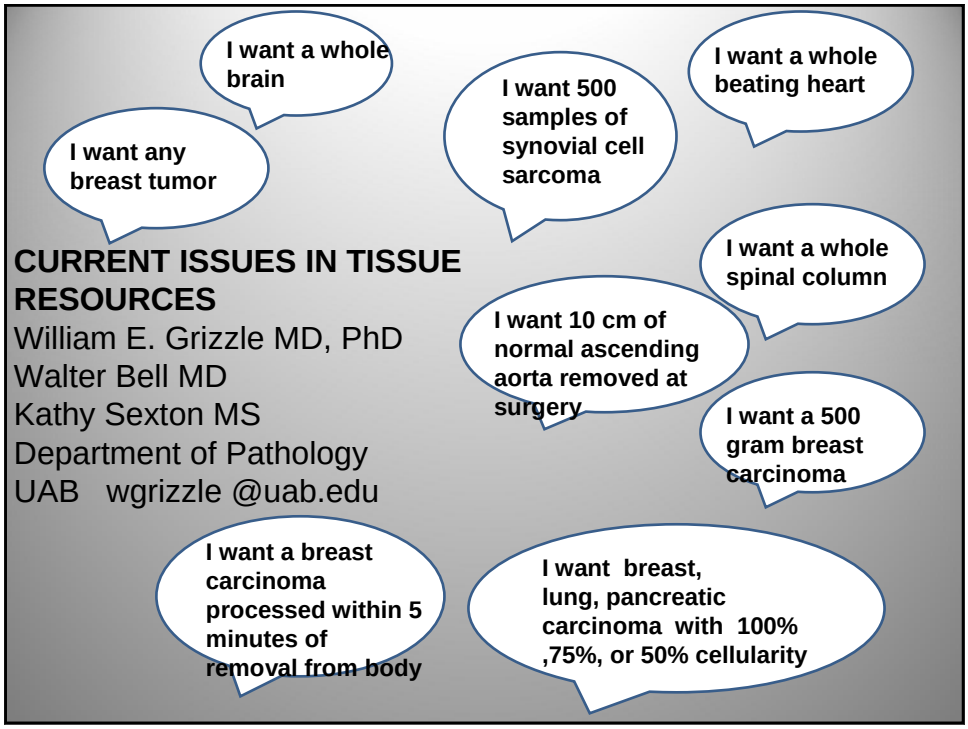
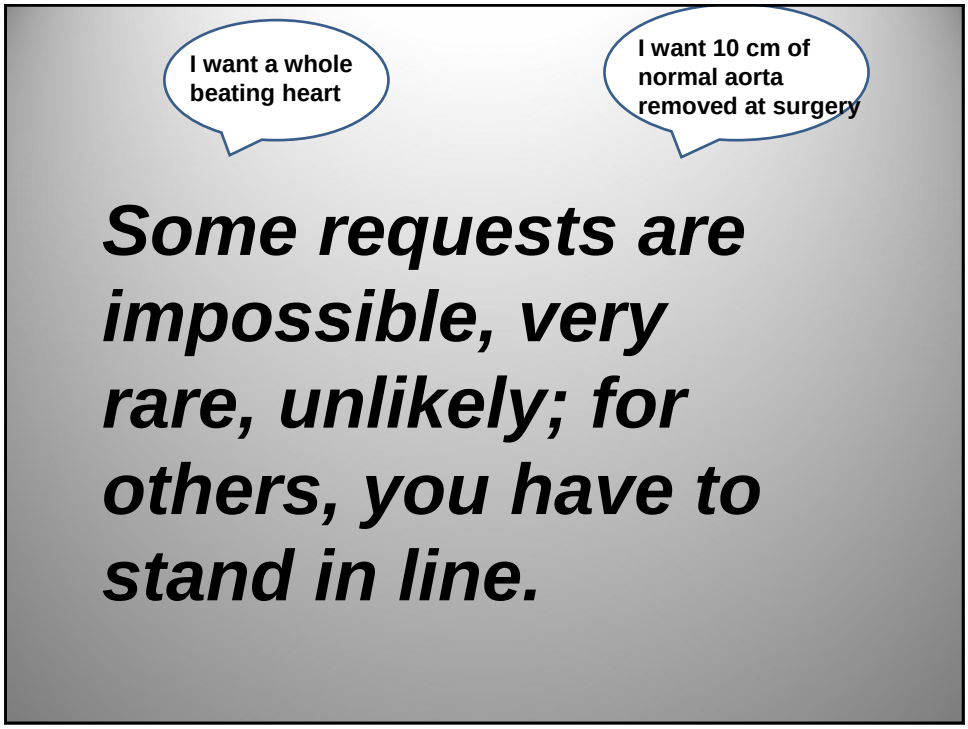
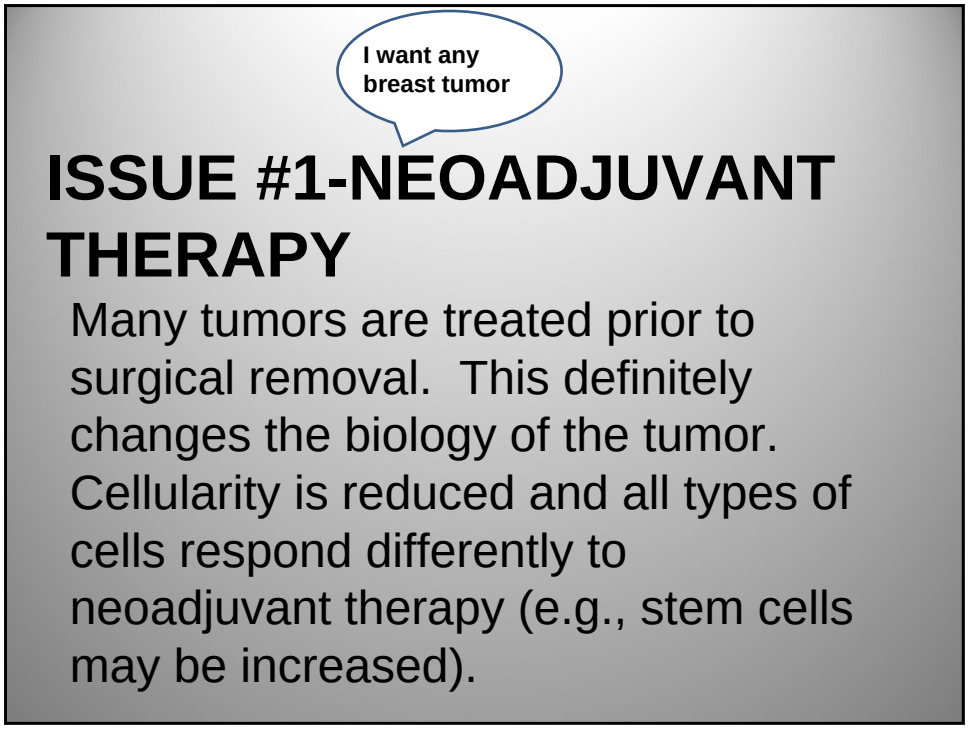


CURRENT ISSUES IN TISSUE RESOURCES

William E. Grizzle MD, PhD
Walter Bell MD
Kathy Sexton MS
Department of Pathology
UAB wgrizzle @uab.edu

Frequently a request for tissue is not understood by the requestor at the time it is made! Try to discuss with investigator to make the request "real."


I want a whole beating heart
I want 10 cm of normal aorta removed at surgery
Some requests are impossible, very rare, unlikely; for others, you have to stand in line.

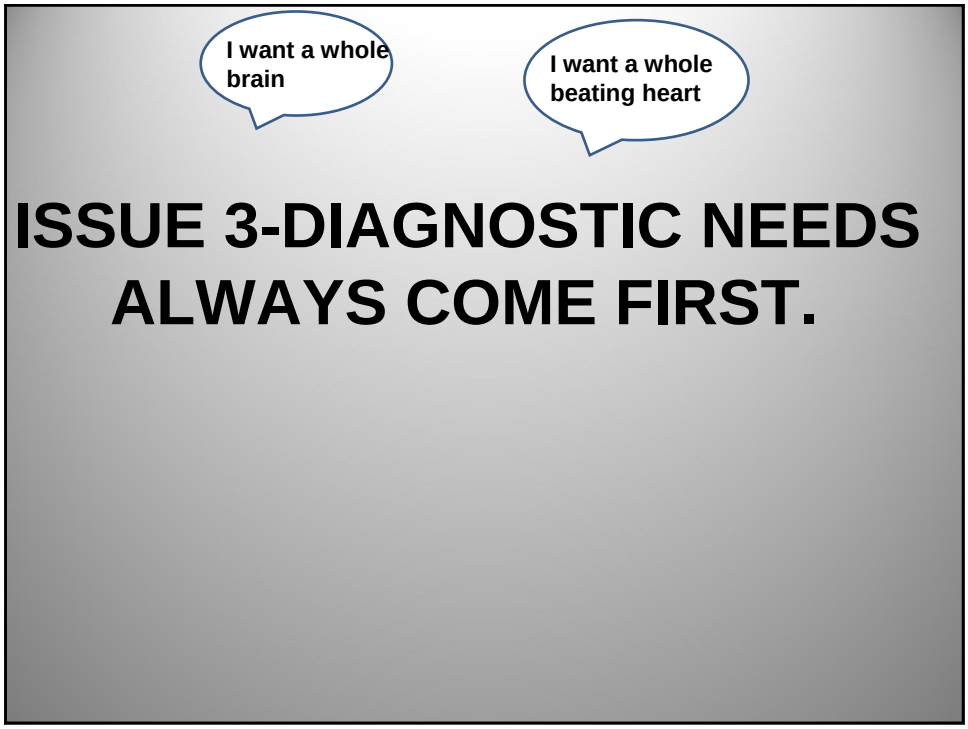

I want any breast tumor
ISSUE #1-NEOADJUVANT THERAPY
Many tumors are treated prior to surgical removal. This definitely changes the biology of the tumor. Cellularity is reduced and all types of cells respond differently to neoadjuvant therapy (e.g., stem cells may be increased).

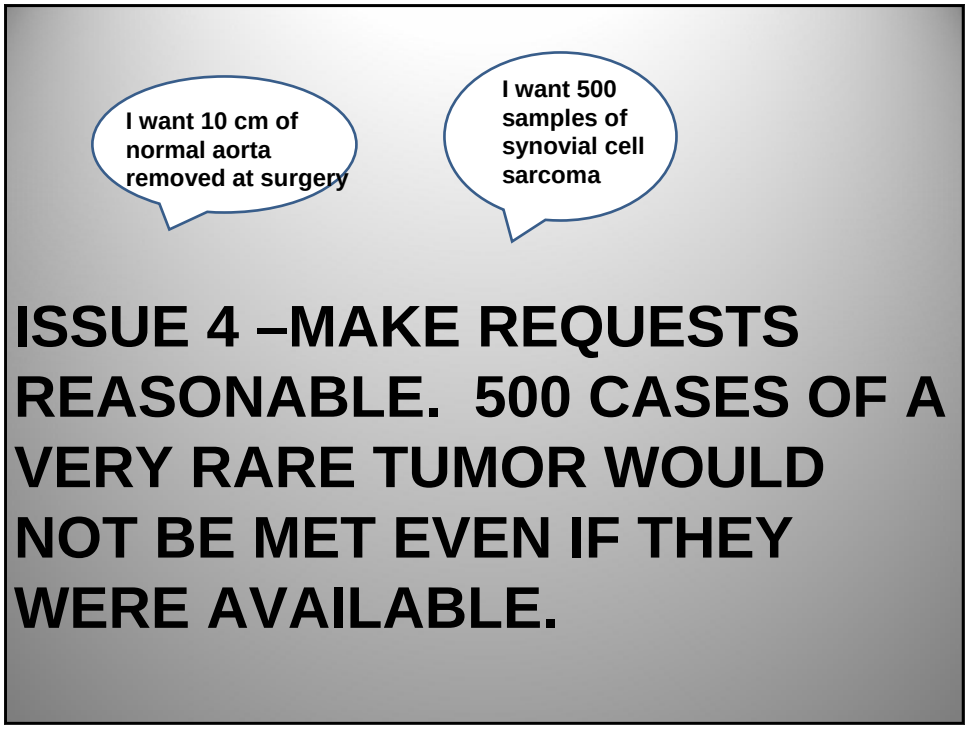
I want any breast tumor

ISSUE #1b- REQUESTS SHOULD BE SPECIFIC.
"TUMOR" BENIGN? SARCOMA? DUCTAL?

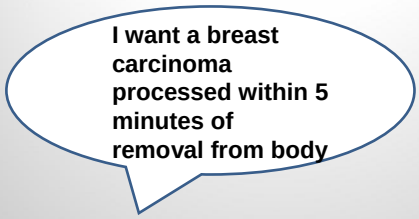
I want a 500 gram breast carcinoma

ISSUE 2-WITH BETTER SCREENING, TUMORS ARE SMALLER OR MAY BE *IN SITU* CARCINOMAS; SAMPLES FOR RESEARCH USUALLY ARE NOT AVAILABLE FROM SMALL TUMORS OR *IN SITU* DISEASE.
REQUESTS FOR LARGE SAMPLES USUALLY ARE MET ONLY AFTER REQUESTS FOR SMALL SAMPLES ARE FILLED-MAKE YOUR METHODS MICRO-.


I want a whole brain
I want a whole beating heart
ISSUE 3-DIAGNOSTIC NEEDS ALWAYS COME FIRST.


I want 10 cm of normal aorta removed at surgery
I want 500 samples of synovial cell sarcoma
ISSUE 4 –MAKE REQUESTS REASONABLE. 500 CASES OF A VERY RARE TUMOR WOULD NOT BE MET EVEN IF THEY WERE AVAILABLE.

ISSUE 5-WHILE TISSUE MIGHT BE AVAILABLE THE EFFORT NEEDED TO OBTAIN THE TISSUE MIGHT NOT BE.



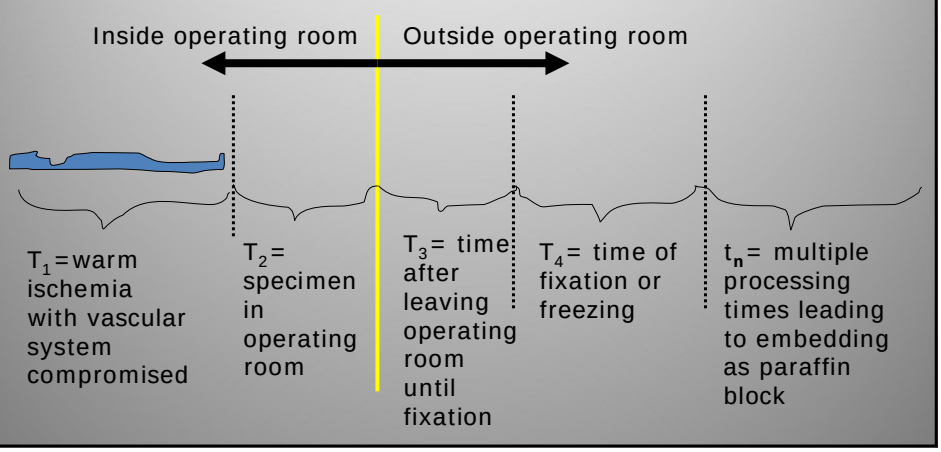
ISSUE 6- IT TAKES TIME TO PROCESS TISSUES; ABOUT 15-20 MINUTES ARE REQUIRED FOR JUST 1 OR 2 ALIQUOTS.

IS RAPID PROCESSING REALLY NECESSARY FOR YOUR STUDY.

ISSUES IN TISSUE RESOURCES

Processing

Time from removal from patient until tissue is embedded in a paraffin block or frozen



WHAT DO WE KNOW ABOUT mRNA AND TIME AFTER TISSUE REMOVAL FROM THE BODY?
NEEDLE BX VS SURGICAL REMOVAL

For surgical removal there is warm ischemia while the organ has its vascular system compromised at body temperature. Probably most genes with unstable mRNA decay during this period.

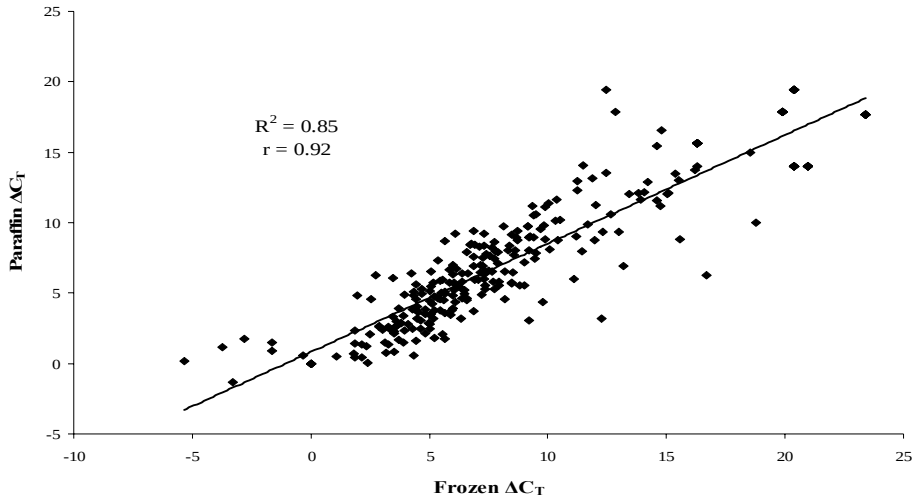
For surgical removal, 20-30 % of genes have increased expression in 1st hour after removal of tissue from the body due to stress genes. Most other genes do not change their expression. If mRNA is intact immediately after surgery , in most cases, it remains intact but may decline in quality as measured by RIN. The hope is the quality is adequate.

Special cases- prostates removed robotically

At least 1 hr added to warm ischemia time compared to non-robotic surgery.

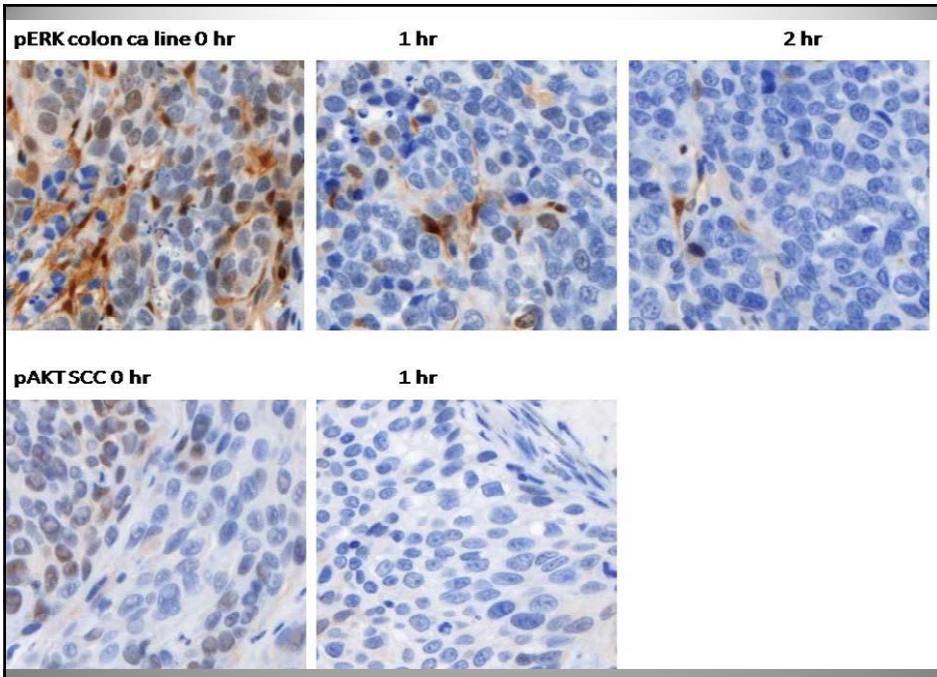
Is the mRNA useful?

Correlation in total RNA isolated from snap frozen vs paraffin embedded ovarian endometriod carcinomas (#7) in the MA-RT-Q-PCR analysis of 90 genes of Wnt-hedgehog pathway or related



BIAS IN TISSUE RESOURCES

Many proteins may degenerate after tissue is removed from the body; of special concern are phosphoproteins perhaps due to the action of phosphatases.



Courtesy Michael Feldman- U Penn.

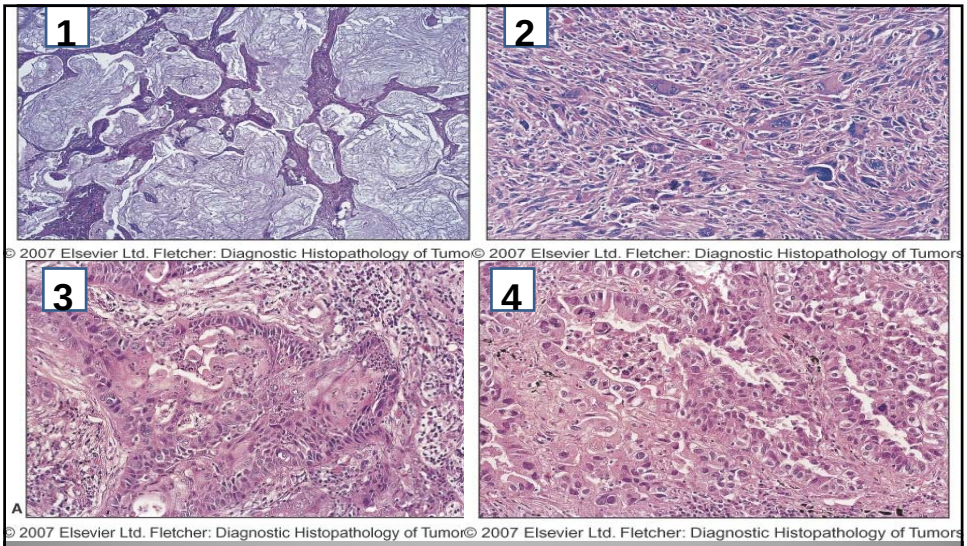
Ischemic time (Animal models, human xenografts)

	Ischemic time (hrs)			Stdev/ave
Antigen	0	1	2	
pERK	0.335	0.213	0.201	30%
Ki67	0.380	0.342	0.352	5%
pAKT	0.156	0.000	0.000	173%
pS6	0.348	0.320	0.320	5%
PStat3	0.336	0.299	0.296	7%
pEGFR	0.298	0.316	0.313	3%

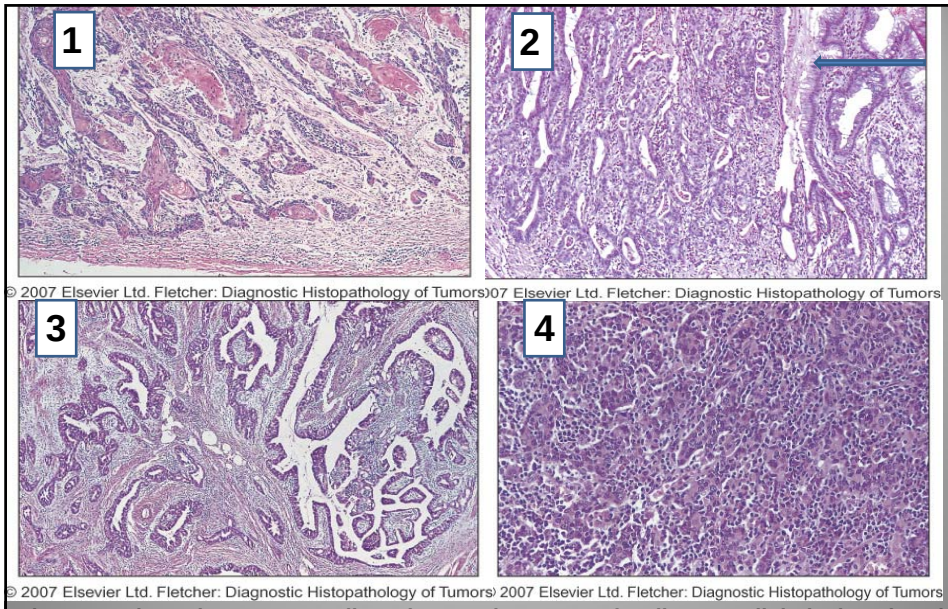
Provided by Michael Feldman at U. Penn

I want breast, lung, pancreatic carcinoma with 100% ,75%, or 50% cellularity

ISSUE 7-MANY REQUESTS DO NOT FOLLOW THE BIOLOGY OF THE DISEASE - UNDERSTAND THE PATHOLOGY-MANY TUMORS DO NOT GROW WITH THIS LEVEL OF CELLULARITY



These are four examples of lung tumors taken from "DIAGNOSTIC PATHOLOGY OF TUMORS 3RD EDITION BY FLETCHER ET AL. The first example is a typical mucinous tumor; all the cellularity is malignant but the AREA of malignant cells is reduced by mucin. In the second and 4th examples, the AREA OF TUMOR is reduced by stroma and/or necrosis. In the 3rd example the area of malignant cells is about 50%; however the inflammatory cells in the stroma may exceed the number of malignant cells.



1 is an esophageal squamous cell carcinoma whose area of malignant cellularity is reduced to less than 50 % by stroma and keratin. 2 is an esophageal adenocarcinoma (arrow points to tumor) whose area is reduced by stroma as is the gastric carcinoma #3. The gastric medullary carcinoma in 4 has more lymphocytes in the stroma than malignant cells of the overall tumor.

I want breast, lung, pancreatic carcinoma with 100% ,75%, or 50% cellularity

I want a breast carcinoma processed within 5 minutes of removal from body

I want a 500 gram breast carcinoma

ISSUE 8- STRICT REQUIREMENTS MAY INDUCE BIAS; BIAS MAY BE CAUSED BY MANY FACTORS SIZE, CELLULARITY, STAGE, GRADE

BIAS IS ESPECIALLY IMPORTANT IN BODILY FLUIDS.

BIAS IN TISSUE RESOURCES

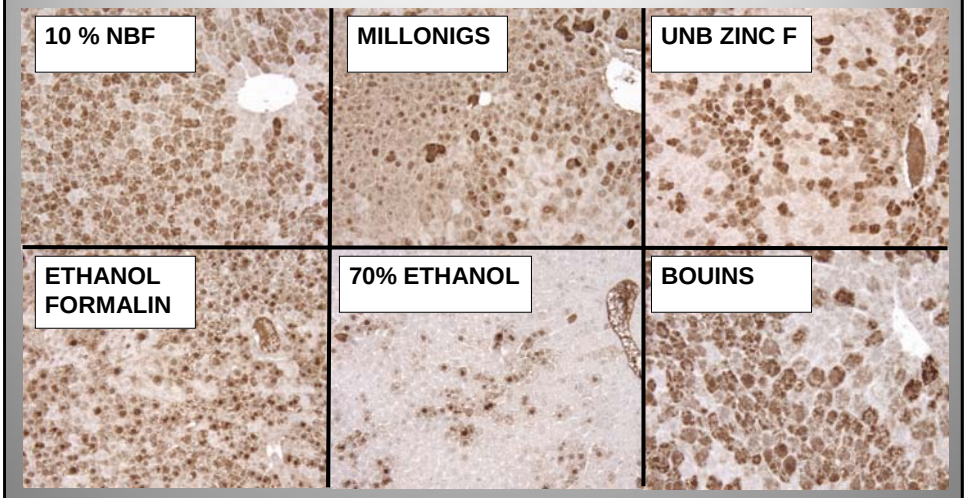
Potential Sources of Bias
Population (age, race, sex)
Patient/Control- Homeostasis
Fasting/Stress/Co-morbidity
Sample Collection- Times Involved
Sample Processing –Type, Volume,
Time of Fixation, Processor
Variables Storage of Blocks -
Length, Temp Slides -Length, Temp
Storage Methods
Standard Operating Procedures (SOPs)
among sites.

BIAS IN TISSUE RESOURCES

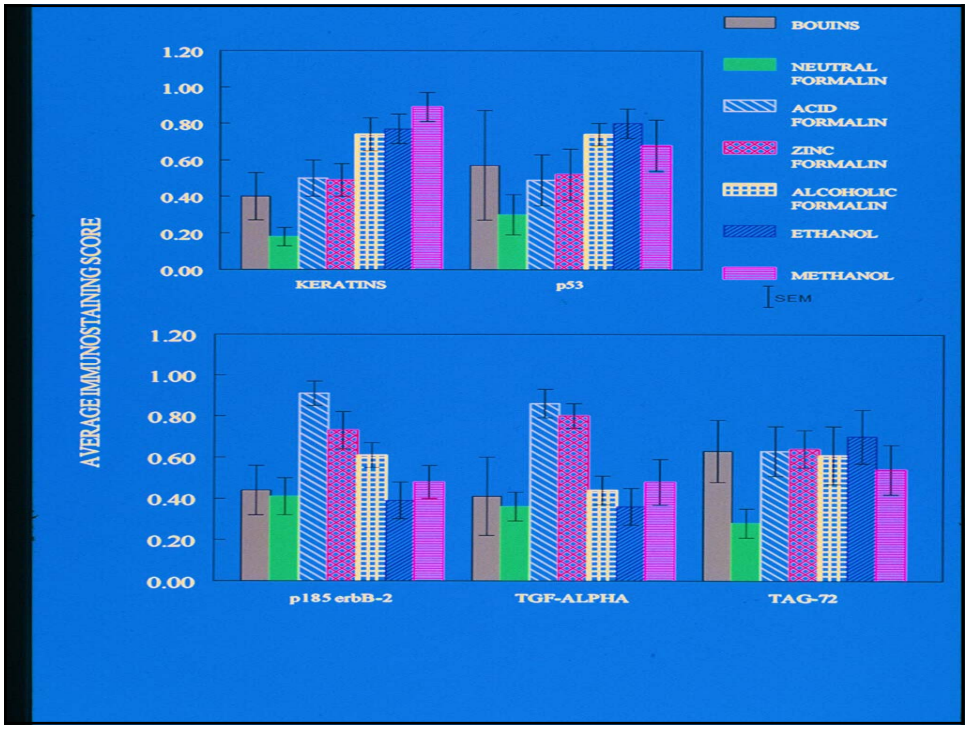
Paraffin Processed
Solid Tissues -Uses
- Biology of Disease
- Progression
- Diagnosis
- Prognosis
- Prediction
- Early Detection
- Surrogate Endpoints
- Targets for Therapy
- Tumor Subcategorization

Paraffin Processed
Solid Tissues –Methods
- Immunohistochemistry
- Histochemistry
- *In Situ* Hybridization
- RT-PCR
- Ultra Structural Analysis
- Cytomorphometric Analysis

EFFECT OF DIFFERENT FIXATIVES ON DEMONSTRATION OF CMV TRANSFECTION IN THE SAME LIVER OF A MOUSE



Note both the patterns and intensity change

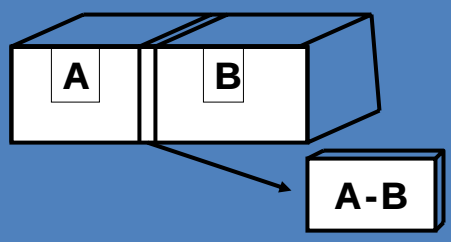


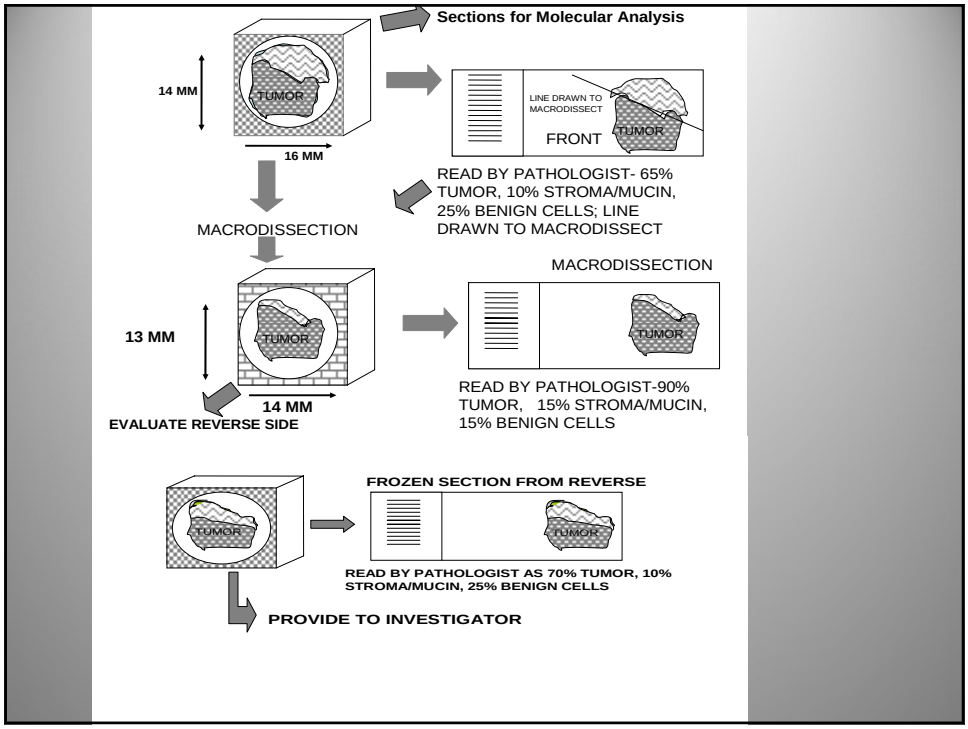
ISSUE 9-Is quality control necessary?

With experience, UAB rejects about 15% of diagnoses of cases. Without experience, up to 40% of cases may be in error.

QUALITY CONTROL

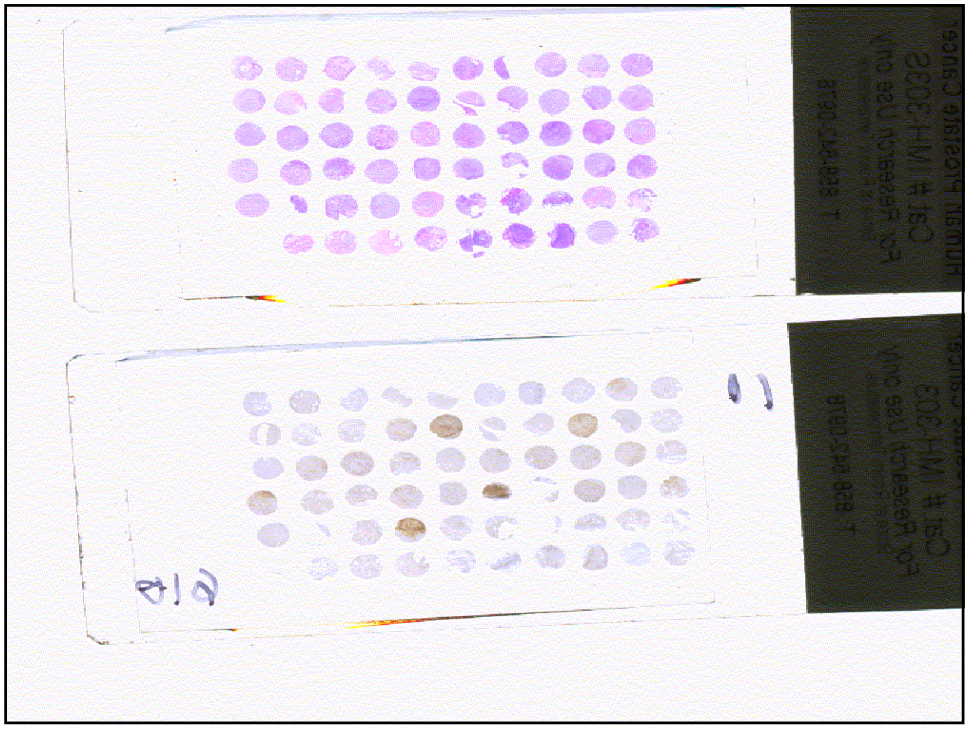
Standard practice





Issue 10-Are tissue arrays better than individual sections in testing molecular markers?

1) Staining 3 cores from one block represent a statistical sample of the tumor.
2) cores at same time.
3) Saves tissue, effort, and resources.
4) Must include controls; faster evaluation.
5) More noise; not more accurate



I want a whole brain

I want any breast tumor

I want 500 samples of synovial cell sarcoma

I want a whole beating heart

I want a whole spinal column

I want 10 cm of normal ascending aorta removed at surgery

I want a 500 gram breast carcinoma

I want a breast carcinoma processed within 5 minutes of removal from body

I want breast, lung, pancreatic carcinoma with 100% ,75%, or 50% cellularity

CURRENT ISSUES IN TISSUE RESOURCES

William E. Grizzle MD, PhD
Walter Bell MD
Kathy Sexton MS
Department of Pathology
UAB wgrizzle @uab.edu