BRN Tissue Acquisition Variables Project

Helen M. Moore, PhD

January 24, 2012

NATIONAL CANCER INSTITUTE

Multiple pre-analytical factors can affect the molecular integrity of the biospecimen

OBBR Office of Biorepositories and Biospecimen Research

Factors (examples):

- Antibiotics
- Other drugs
- Type of anesthesia
- Duration of anesthesia
- Arterial clamp time

Time 0

Factors (examples):

- Time at room temperature
- Temperature of room
- Type of fixative
- Time in fixative
- Rate of freezing
- Size of aliquots

Patient	Medical/ Surgical Procedures	Acquisition	Handling/ Processing	Storage	Distribution	Scientific Analysis	Restocking Unused Sample

Pre-acquisition | **Post-acquisition**

The Lifecycle of the Biospecimen

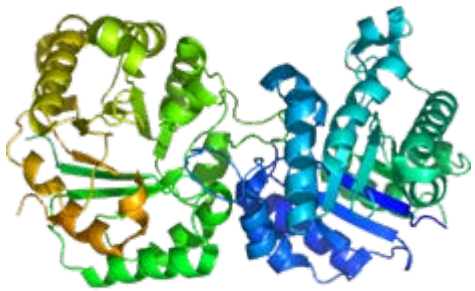
How Can Changes in Molecular Integrity of Biospecimens Affect Molecular Readout?

OBBR Office of Biorepositories and Biospecimen Research

Genomics

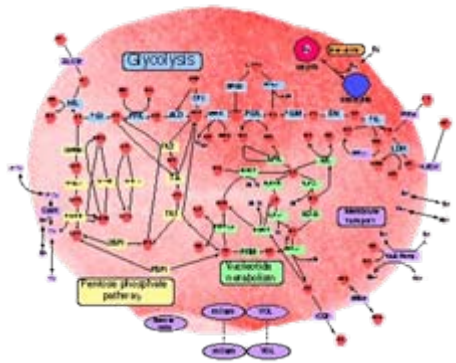
Changes in specific transcript levels based on ischemic time, not disease

Proteomics



- Lack of reproducibility of protein biomarkers in discovery research
- Inconsistent IHC results in Research and Clinical Labs

Metabolomics

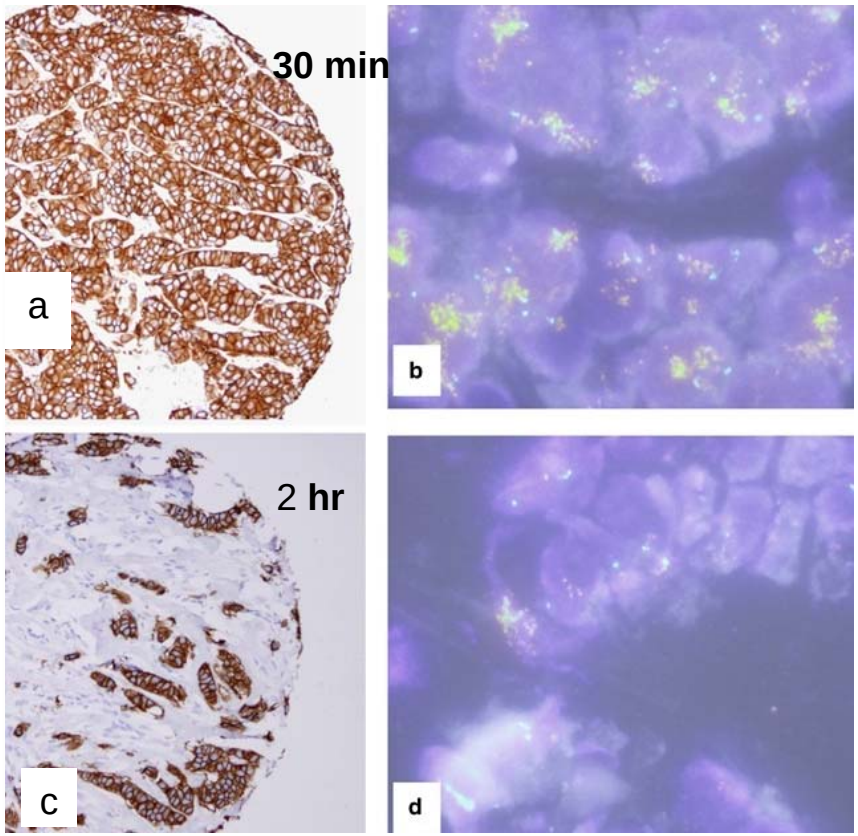


Inconsistencies in small molecule readouts, yielding results that point to the wrong pathway

FFPE:
Delay to Fixation and Molecular Assay Results

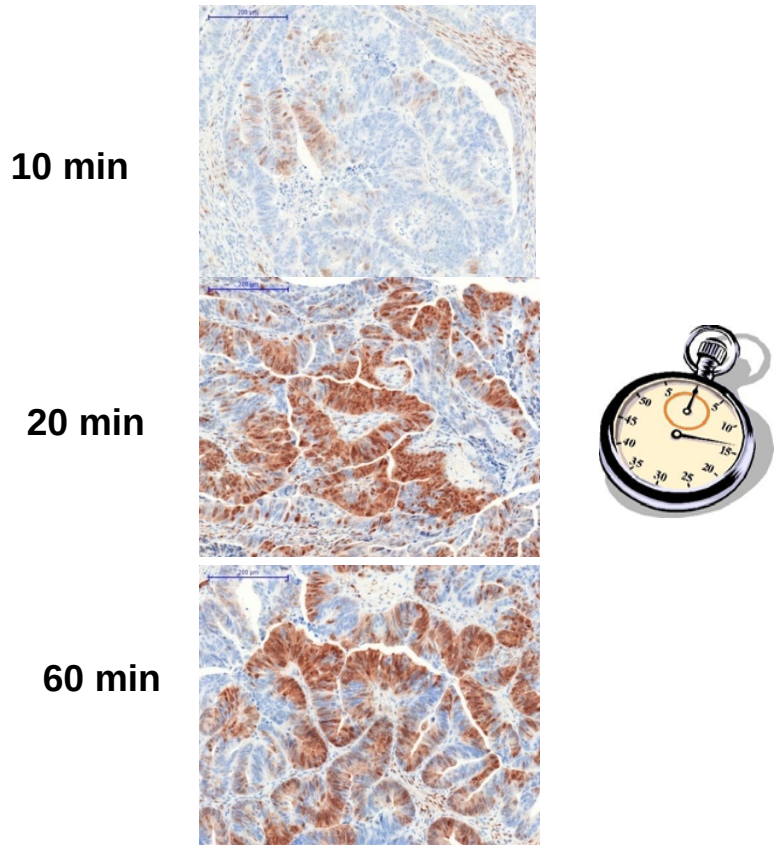
OBBR Office of Biorepositories and Biospecimen Research

HER2 IHC and FISH in Breast Cancer:
Loss of Biomarker Signal with Time to Fixation



Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

Phosphoprotein pMAPK IHC of Colon Cancer :
Gain of Biomarker Signal with Time to Fixation



Hartmut Juhl, Indivumed GmbH, BRN

BRN Project in Tissue Acquisition and Processing Variables

OBBR Office of Biorepositories and Biospecimen Research

-Post-operative ischemia
-Room temperature
-Type of preservative
-Rate of freezing/fixing

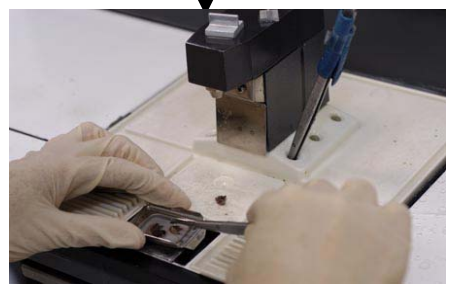
Tissue processing
-Multiple formulaic variables
- Multiple time settings for each

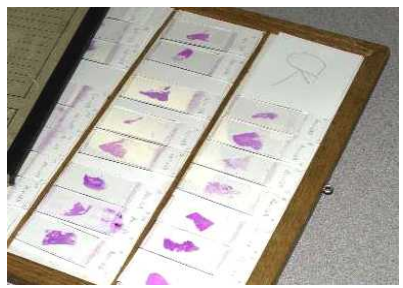
Recorded/annotated
-Anesthesia
-Intra-operative ischemia
-Many other variables

2 hospital centers

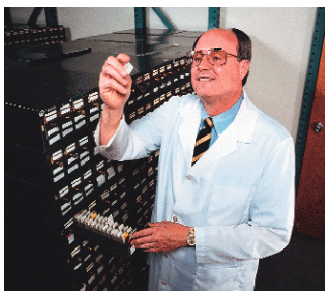
Cancer and normal tissues

Systematically alter selected variables





H&E
IHC
FISH
RNA isolation
Storage



BRN Scientific Steering Committee and Subcommittees

OBBR Office of Biorepositories and Biospecimen Research

- **Interdisciplinary Committee**
  - Pathologists, Statisticians, Scientists, Informaticist, Patient Advocate
- **Working Group Subcommittees**
  - IHC/FSH
  - DNA/RNA
- **Subcommittees developed plans for:**
  - Markers to be tested
  - Processes and procedures for testing markers
  - Data output desired
  - Data Analysis

Experimental Design Overview

OBBR Office of Biorepositories and Biospecimen Research

- Goal: to understand how variability in tissue fixation and processing affects the molecular integrity of the resulting tissues
- Approach: treat tissue fixation and processing (FFPE) as a manufacturing process which we want to study, but over which we do not have complete control
- Identify key preanalytical factors that expert intuition says are of most importance to getting a “good” biospecimen in the clinic
- Study these key factors in a first set of experiments to test expert intuition
- Evaluate experimental results and then plan the next set of experiments

Experimental Design Overview

OBBR Office of Biorepositories and Biospecimen Research

- **2 key preanalytical factors identified for first set of experiments:**
  - Delay time to fixation
  - Time in fixative before processing
- **4 time points for each of these two factors identified**

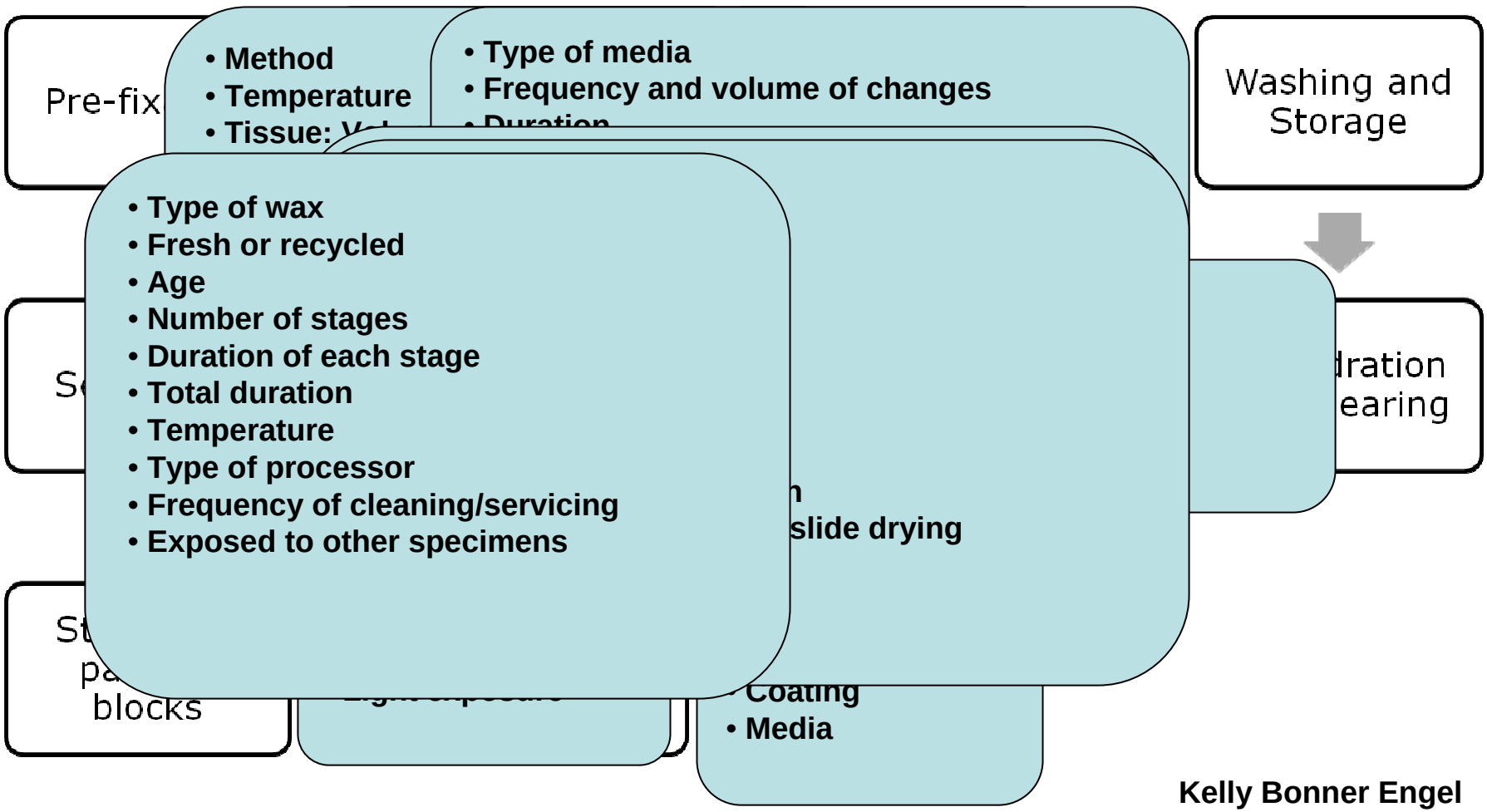
Some of the Challenges Addressed

OBBR Office of Biorepositories and Biospecimen Research

- Development of biospecimen lifecycle Common Data Elements (CDEs) and data enty system
- Operational schemes for labeling and randomization
- Experimental design and execution

Developing Common Data Elements for Fixation and Processing Parameters

OBBR Office of Biorepositories and Biospecimen Research

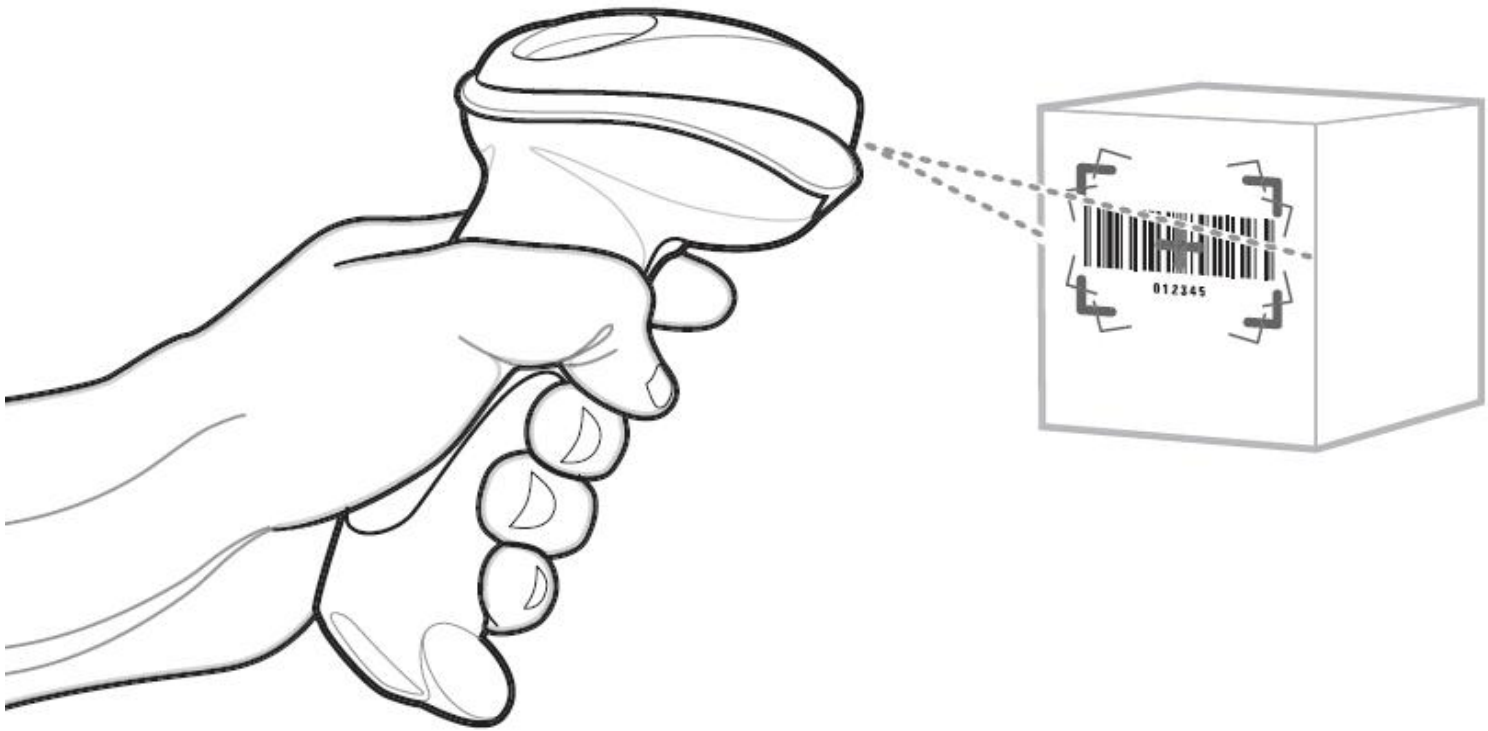


Kelly Bonner Engel

OpenClinica utilized for Data Collection

OBBR Office of Biorepositories and Biospecimen Research

- **Barcode reader functionality added – records a timestamp when reading barcode**



Biospecimen ID's, labels, Experimental Keys

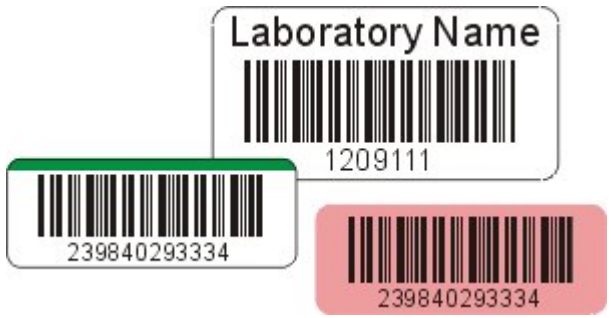
OBBR Office of Biorepositories and Biospecimen Research

- **Van Andel Research Institute (VARI)**
  - caHUB contracted Comprehensive Biospecimen Resource
  - Prepare ID's, labels, and randomization tool ("Experimental Key") for BRN
  - Perform basic molecular analysis for BRN
  - Manage shipment and storage of BRN biospecimens

Biospecimen ID's, labels, Experimental Keys

OBBR Office of Biorepositories and Biospecimen Research

- **IDs and labels:**
  - BRN case IDs: umbrella ID for all biospecimens collected at one event (one donor on one day)
  - BRN biospecimen IDs: all derivative biospecimens from that case
  - Random numbers
  - Numbers and barcodes placed on labels



Biospecimen ID's, labels, Experimental Keys

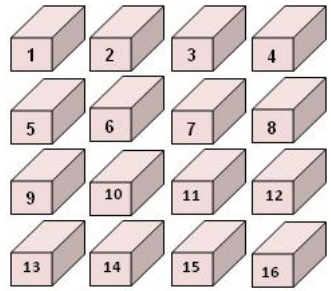
OBBR Office of Biorepositories and Biospecimen Research

- **IDs and labels:**
  - In most cases, labels are placed on collection and storage containers at the sites
  - FFPE cassettes are pre-etched with (random number) biospecimen IDs at VARI

Biospecimen ID's, labels, Experimental Keys

OBBR Office of Biorepositories and Biospecimen Research

- **Laminated Grids**
  - To aid in dissecting tissue into aliquots
- **Experimental Keys:**
  - To randomly assign aliquots to a particular experimental protocol



Scannable Key ID

Experimental Protocol	Grid Location
A	9
B	13
C	5
D	10
E	2
F	7
G	16
H	1
I	11
J	4
K	8
L	14
M	3
N	12
O	6
P	15

How ID's, labels, Experimental Keys work with OpenClinica

OBBR Office of Biorepositories and Biospecimen Research

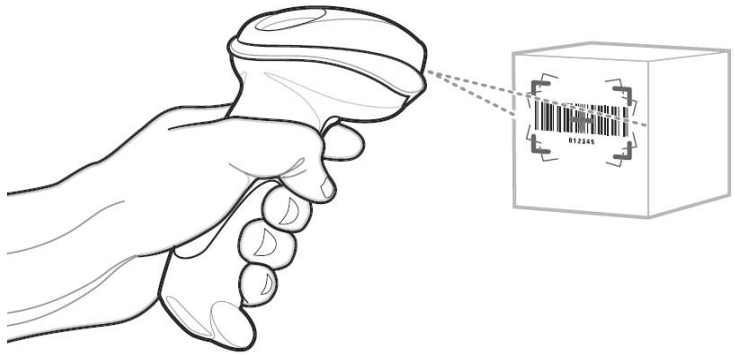
- Technician scans each biospecimen label into the OpenClinica system
- A date and time stamp populates the appropriate OpenClinica data entry field
- The layout of the Experimental Key is aligned with the OpenClinica data entry form

View Section Data Entry for BRN Tissue Processing Worksheet4 Original

CRF Info

BRN Tissue Processing Worksheet4 Original	Discrepancy Notes: New, Updated, Resolution Proposed, Closed, Not Applicable
Study Subject ID:	Person ID:
Study/Site:	Age At Enrollment:
Event: ()	Date of Birth: Sex:
Interviewer Name: *	Interview Date: *

Press the little flag icon beside an input to enter discrepancy notes, please note that you can only save the notes if CRF data entry has already started.



Exit

Tissue ...(0/71) -- Select to Jump --

Title: Tissue Processing Worksheet

Case Information

caHUB/BRN Case ID (OC Study Subject ID)	BSS Tissue Bank Participant ID (OC Secondary ID)	BRN parent sample barcode ID from which required study samples were derived	Experimental Key Barcode ID	Date and time Experimental Key Barcode ID was scanned	
CA-12345–BRN		12345678-BRN	KE -123456-BRN	2011-09-28T12:02:00	X

ADD

Frozen Tissue Sample Information (Bottom Section of Tumor Block)

Scanned ID of frozen tumor tissue cryosette (bottom section of tumor block)

Time that tumor tissue sample was frozen in liquid nitrogen

Planned Delay to Fixation 0-30 Minutes Experimental Protocol A-D and Top FFPE Section Part I

Experimental Protocol A-D And Top FFPE Section	Planned delay to fixation time	Scanned ID of cassette: Record first scan	Time that cassette was first scanned	Scanned ID of cassette: Record time placed in fixative	Time that cassette was placed into fixative (Scanned)	OPTIONAL: Calculated actual delay to fixation time	
Protocol A: <6 hours in fixativ	0-30 minutes	12345678-BRN	2011-09-28T12:35	12345678-BRN	2011-09-28T17:45:00		X
Select	0-30 minutes						X
Select	0-30 minutes						X
Select	0-30 minutes						X
Select	0-30 minutes						X

ADD

Scannable Key ID

Experimental Protocol	Grid Location
A	9
B	13
C	5
D	10
E	2
F	7
G	16
H	1
I	11
J	4
K	8
L	14
M	3
N	12
O	6
P	15

Tissue Processor

OBBR Office of Biorepositories and Biospecimen Research

- **Leica Peloris**
- **Can perform multiple runs in parallel**
  - So we can do a 6 pm and a midnight run on the same machine
  - One machine to maintain and QC
- **Uses standard reagents**
  - No need for Leica-branded consumables
  - Can do isopropanol and xylene-free runs later in project, if desired
- **Reagents for different runs are pulled from the same “bucket” of reagent**
  - One set of stepwise reagents to maintain and QC

Step	Reagent type	Reagent group	Time (min)	Temp (°C)	P/V	Stirrer	Drip time (s)
1	Formalin	Fixatives	20	45 ??	Ambient	Medium	10
2	Ethanol	Dehydrants	20	45	Ambient	Medium	10
3							
4							
5							
6							
7							
8							
9	Xylene	Clearers	30	45	Ambient	Medium	10
10	Xylene	Clearers	60	45	Ambient	Medium	10
11	Paraffin wax	Wax	40	65	Vacuum	Medium	10
12	Paraffin wax	Wax	40	65	Vacuum	Medium	10
13	Paraffin wax	Wax	60	65	Vacuum	Medium	10
Processing time			8:08:00				

ER recommendations, 2008: It is strongly recommended that none of the tissue processor solutions, excluding paraffins should exceed 37 C if the processor contains breast tissue for potential ER and other biomarker testing. If such heated processors are used, then the laboratory director is responsible for validation of ER results against parallel samples that have been processed conventionally without excess heat.
(Temperatures above 37C are not recommended for tissue that will be IHC stained.)

Components need to collect the first tissues: Experiment 1

OBBR Office of Biorepositories and Biospecimen Research

- ✓ IRB Approvals: UNM and Vanderbilt
- ✓ Project equipment in place (including tissue processor)
- ✓ BRN data collection tool (OpenClinica) tested and ready to go
- ✓ Biospecimen ID's, labels, randomization tools ready to go
- ✓ SOPs and other deployment and training materials complete
- ✓ Internal caHUB approvals processes complete
- ✓ Training conducted late October/early November 2011
- ✓ Currently collecting for Experiment 1 at both sites

Thanks to…

OBBR Office of Biorepositories and Biospecimen Research

- Therese Bocklage
- Carolyn Compton
- Kelly Engel
- Erin Gover
- Howard Greenman
- Kelly Higgins
- Andrea Kelly
- Nicole Lockhart
- Cathleen Martinez
- Marlena Martinez
- Neil Mucci
- Angela Notah-Chavez
- Nancy Roche
- Anna Smith
- David Tabor
- Jake Vargas
- Jim Vaught
- Kay Washington
- Carol Weil
- Kerry Wiles
- Gail Wiseman
- Jennifer Hunt and the BRN Scientific Steering Committee

BRN Tissue Acquisition Variables Project

Helen M. Moore, PhD

January 24, 2012

NATIONAL CANCER INSTITUTE