The Disappearing Line of Demarcation Between Research and Clinical Care: Bridging the Standards Gap

Molecular Data

Biomarkers

Diagnosis / Therapy

DETERMINES QUALITY HERE

PERSONALIZED CANCER CARE

Biospecimen Analysis

Biospecimen Collection

QUALITY HERE

Biospecimen Processing and Banking

Pervasive Standards Deficits Contribute to the Lack of Progress

- Low reproducibility of academic publications
- Poor access to rigorously annotated, fit-for-purpose biospecimens from stringently phenotyped sources
- Insufficient control of pre-analytical parameters
- Variable analytical standards
- Idiosyncratic ‘lab-specific’ analytical methods
- Small studies lacking statistical power
- Chaotic data reporting formats and poor database interoperability
- Poor compliance with journal policies on reporting standards
- Non-existent quality management systems

Sources of Bias in Molecular Marker Research in Cancer

- David F. Ransohoff and Margaret L. Gourlay, 2010

JOURNAL OF CLINICAL ONCOLOGY

Official Journal of the American Society of Clinical Oncology

Table 1. Sources and "Locations" of Bias in Marker Research

Source of Bias	Location of Bias: Before or After Specimens Are Received in the Laboratory: Before	After	Example
Features of subjects, determined in selection: Age Sex Comorbid conditions Medications	X		Cancer subjects are male, whereas control subjects are mainly female. Bias: Assay results may depend on sex.
Specimen collection	X		Cancer specimens come from one clinic, whereas controls come from a different clinic. Bias: Assay results may depend on conditions that differ between clinics.
Specimen storage and handling	X	X	Cancer specimens are stored for 10 years because it takes longer to collect them, whereas control specimens are collected and stored over 1 year. Bias: Assay results may vary with duration of storage, or with different numbers of thaw-freeze cycles.
Specimen analysis		X	Cancer specimens are run on one day, whereas control specimens are run on a different day. Bias: Assay results may depend on day of analysis in a machine that "wanders" over time.

NOTE. The table shows examples of different sources of bias and the location of the bias before or after specimens are received in the laboratory. The list is not exhaustive; other biases may be important, and the biases listed may or may not be important in any given research study, depending on details of biology and technology (ie, what is being measured and how it might be influenced).

Pre-analytical Factors Affect Molecular Composition and Integrity: Data Needed by Biobanks!

Specimen is viable and biologically reactive → **Molecular composition subject to further alteration/degradation**

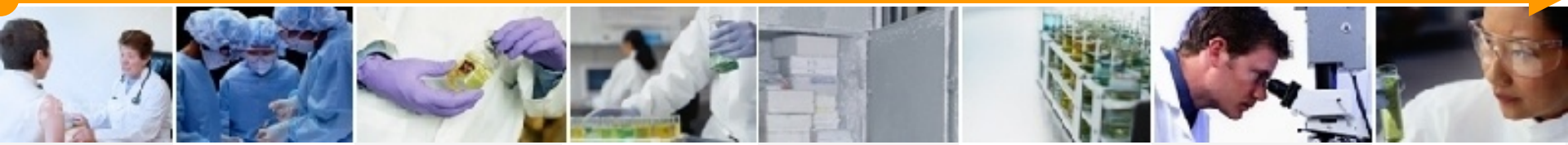
Factors (examples):

- **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**

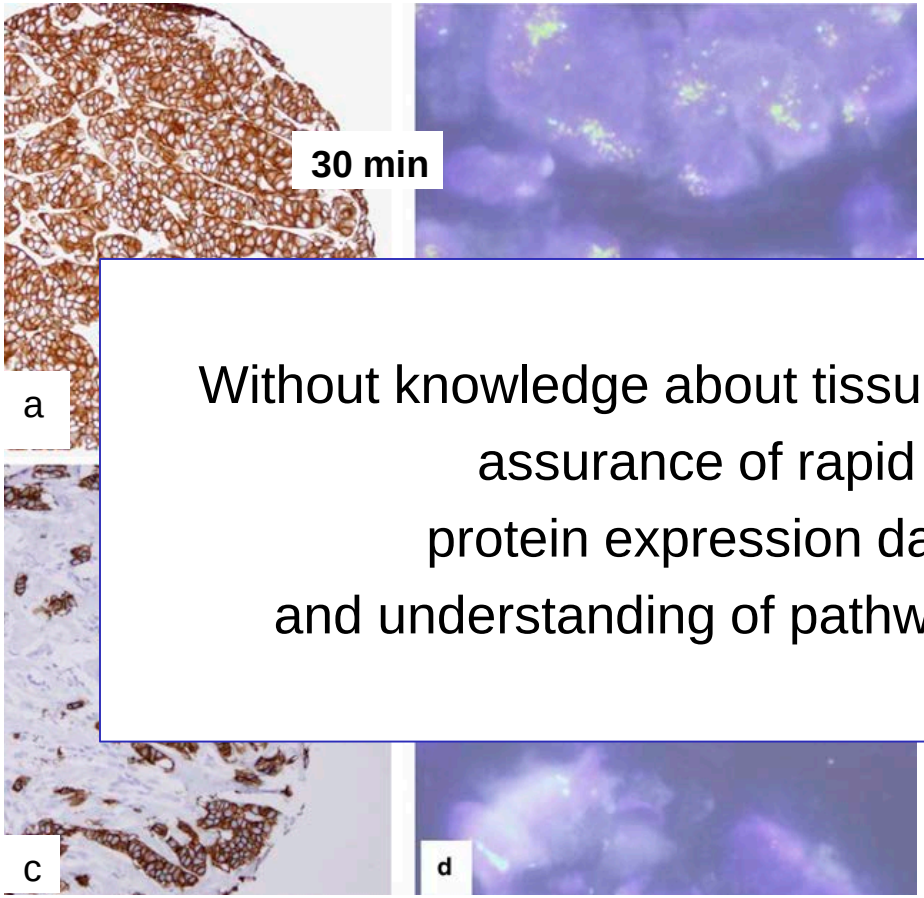


Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage | Distribution | Scientific Analysis | Restocking Unused Sample

Pre-acquisition | **Post-acquisition**

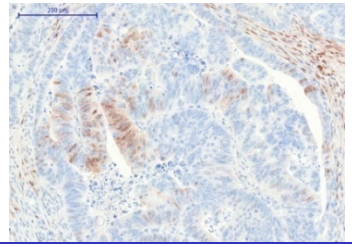
Cold Ischemia and Molecular Assay Results

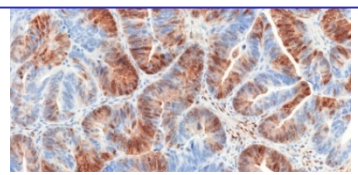
HER2 IHC and FISH in Breast Cancer:
Loss of Biomarker Signal with Time to Fixation



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

10 min



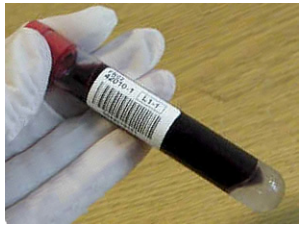


Without knowledge about tissue processing methods and assurance of rapid tissue fixation,
protein expression data are unreliable,
and understanding of pathway activity is impossible

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

Hartmut Juhl, Indivumed GmbH, BRN

Blood Collection and Plasma Processing: Biomarkers and Circulating Tumor Cells



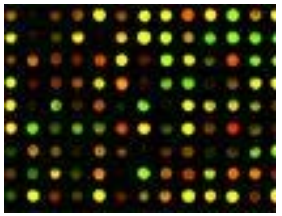
Collection Tubes and Order of draw

Processing Procedure, Temperature and Time

Distribution & Storage

Blood Draw Procedure

Patient Consent and Preparation



Molecular Analysis

Plasma Biomarkers: Protocol Variations with Known Effects on Assay Results

Procedure	Variations
Venipuncture	**Needle gauge** **Priming volumes**
Phlebotomy	**Patient position (seated /reclining)** **Tourniquet time** **Tube orders** **Venipuncture sites**
Collection device	**Tube types**
Blood derivatives and processing	**Anticoagulant types** **Temperatures** **Centrifugation speeds** **Processing time**
Time between collection and storage	**Variable or unknown times**
Storage and shipping	**Temperature** **Duration**

Garbage in…

…Garbage out

Biospecimens as Sources of Biomarkers

What defines biospecimen "quality"?

- **Fit for purpose design**
  - Depends on the stringency / requirements of the analysis to be performed, the specific platform used, and the lability of the molecular species
- **Data-driven standard operating procedures**
  - Biospecimen science needed to generate evidence
- **Verification by quality control procedures and a total quality management system**