# Preanalytical Processing: The Biospecimen Quality Imperative

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**Evolution and Revolution in Anatomic Pathology**
**Banbury Conference Center**
**December 5, 2016**


BioIT World 2011 - by **Sorena Nadaf, M.S. M.MI**

*Director - Translational Informatics, CIO*

# Biospecimen Quality Drives Both Molecular Medicine and Translational Research

Molec

DETERMINES QUALITY HERE

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nagement

**PRECISION MEDICINE**

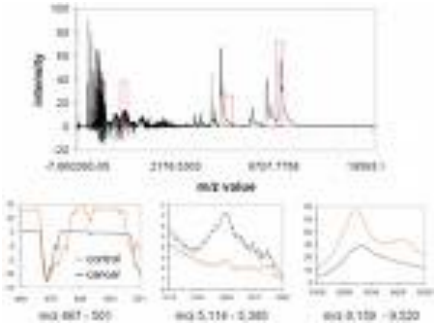
Biospecimen Analysis

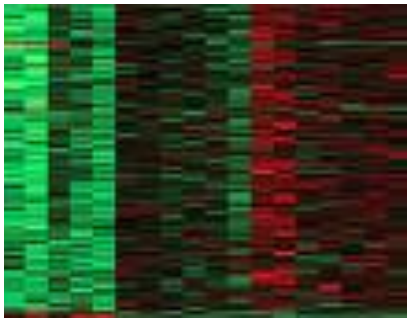
Biospecimen Collection

QUALITY HERE

Biospecimen Handling and Processing

# Biospecimen Quality Impacts Both Clinical And Research Outcomes





**Effects on Clinical Outcomes**

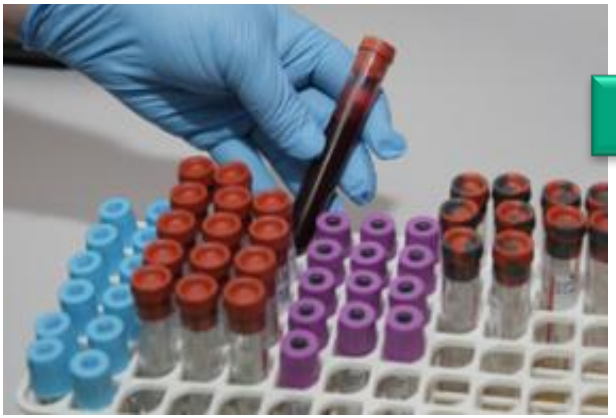
- Potential for incorrect diagnosis
- Potential for incorrect treatment
  - Therapy linked to diagnostic test on a biospecimen

**Effects on Research Outcomes**

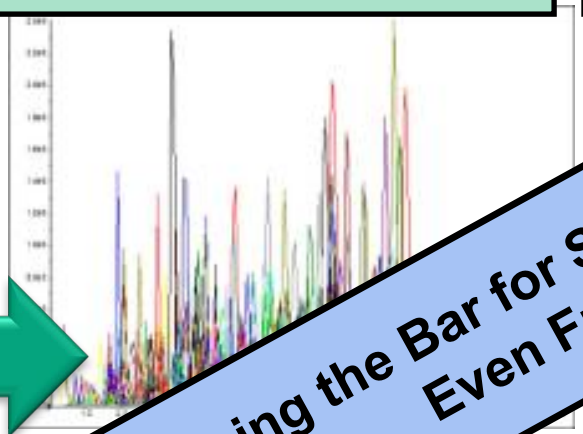
- Irreproducible results
  - Variation in mutation data
  - Variation in gene expression data
  - 11-25%  reproducibility of published biomedical data
- Misinterpretation of artifacts as biomarkers

# Evolution of Biomarker Testing

Unianalyte Tests



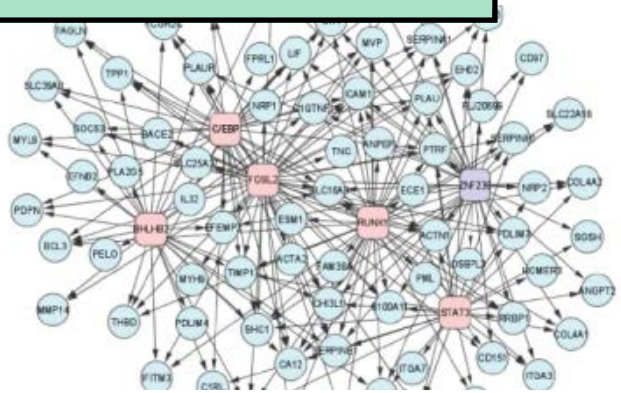
Multianalyte Tests



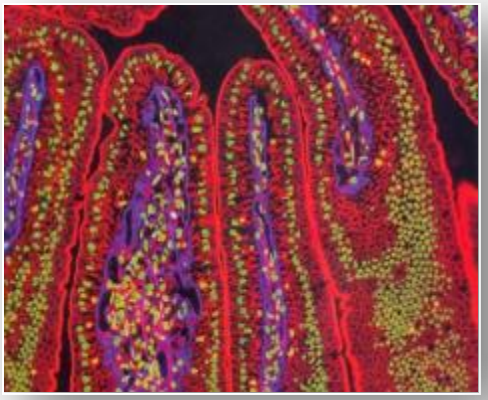
Omics Analyses

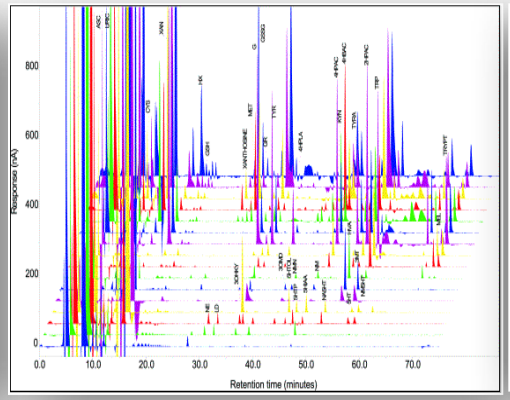
Raising the Bar for Specimen Quality Even Further!

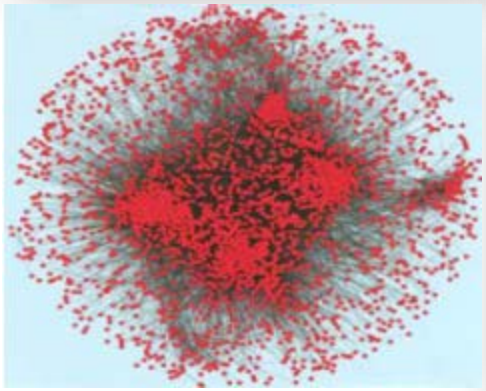
Networks and Systems

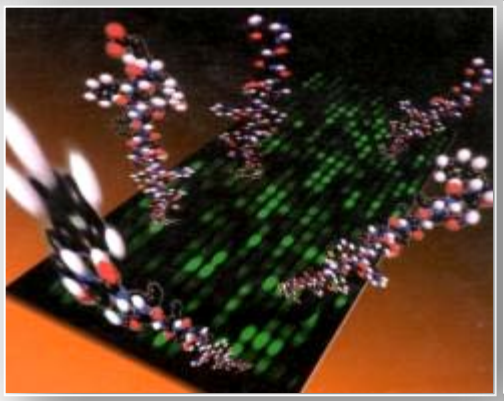


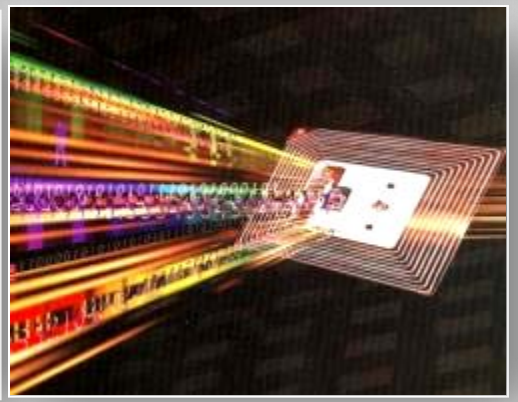
# And It's Getting Far More Challenging









**Biospecimens and Analysis of Molecular Pathway/ Network Perturbations**

**Multiplex Assays and Complex Signal Deconvolution Algorithms**

**Novel Instrumentation, Automation and Large Scale Informatics**

**Patient Profiling, Rational Rx and Health Monitoring**

**Courtesy of G. Poste**

# Powerful Tools: Powerful Risks

- Technology development is exponential, not linear
- Analysis technologies become ever faster, better, cheaper
- No technology can spin straw into gold – you must begin with gold!
  - "Even our technology cannot save a bad sample." – Carrie Browning, Illumina
- The technological capacity exists to produce low-quality data from low-quality analytes with unprecedented efficiency
- We now have the ability to get the wrong answers with unprecedented speed

# Rigor and Reproducibility for Biomarker Measurement in the Lab: How Is It Assured?

- Place where test is done
  - CLIA/CAP laboratory accreditation
- People doing the test
  - Education
  - Proficiency testing
  - Licensure
- Platforms used for testing
  - CDRH approved devices
- Processes followed for testing
  - SOPs
  - Quality management
- Patient samples to be tested
  - Wild West: No requirements to control or document pre-analytics except the ASCO-CAP guidelines for breast cancer

# Pre-analytical Factors Affect Both Molecular Composition and Molecular Quality

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

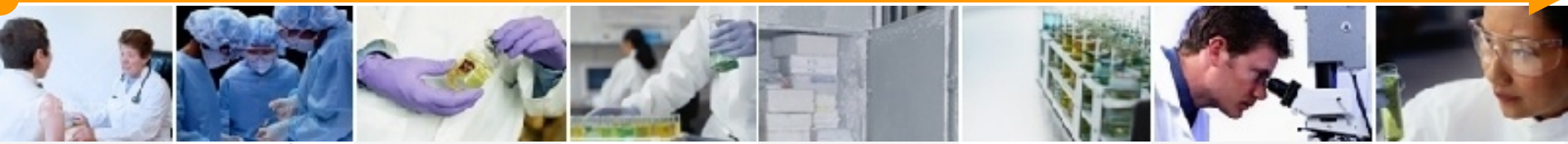
**Time 0**

**Factors (examples):**

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

ANALYSIS

STORAGE



| 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**

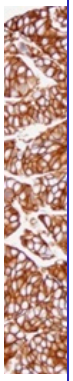
**pMAPK IHC of Colon Cancer :**
**e to**

Without knowledge about tissue processing methods and

assurance of rapid tissue fixation,

protein expression data are unreliable,

and understanding of pathway activity is impossible.



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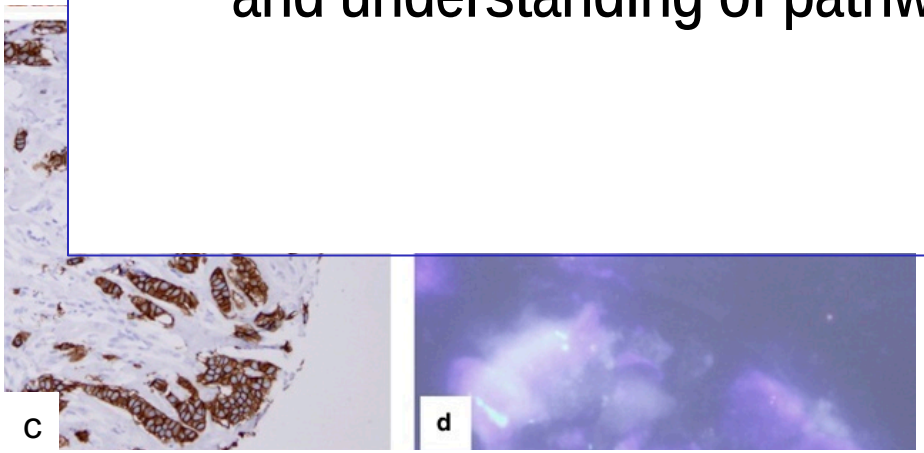

c

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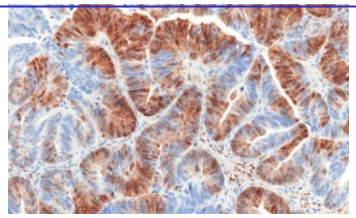
**60 min**

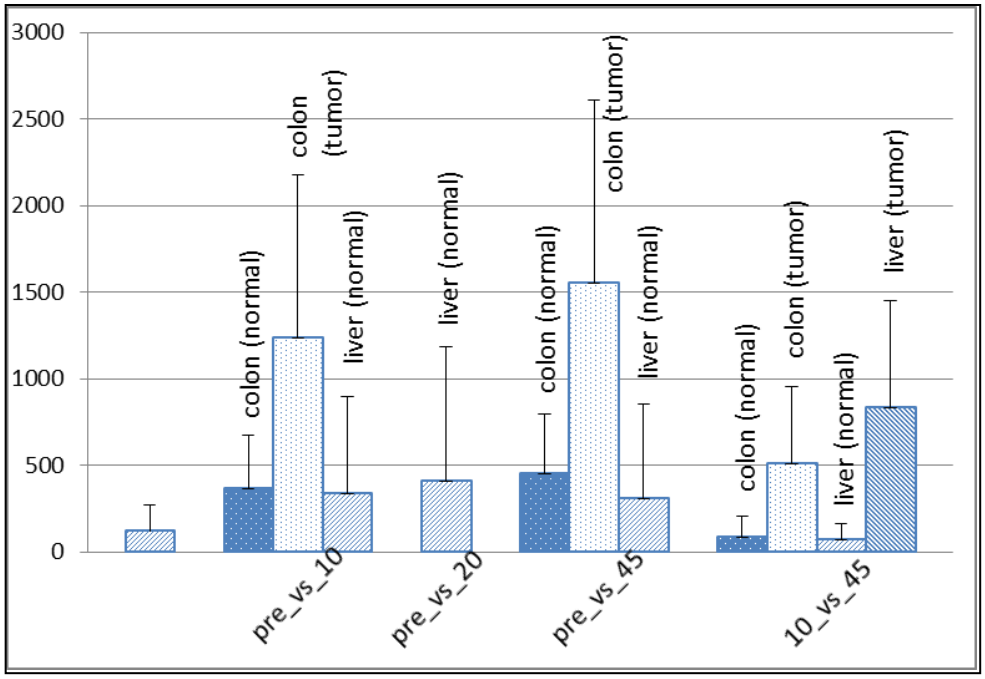


Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

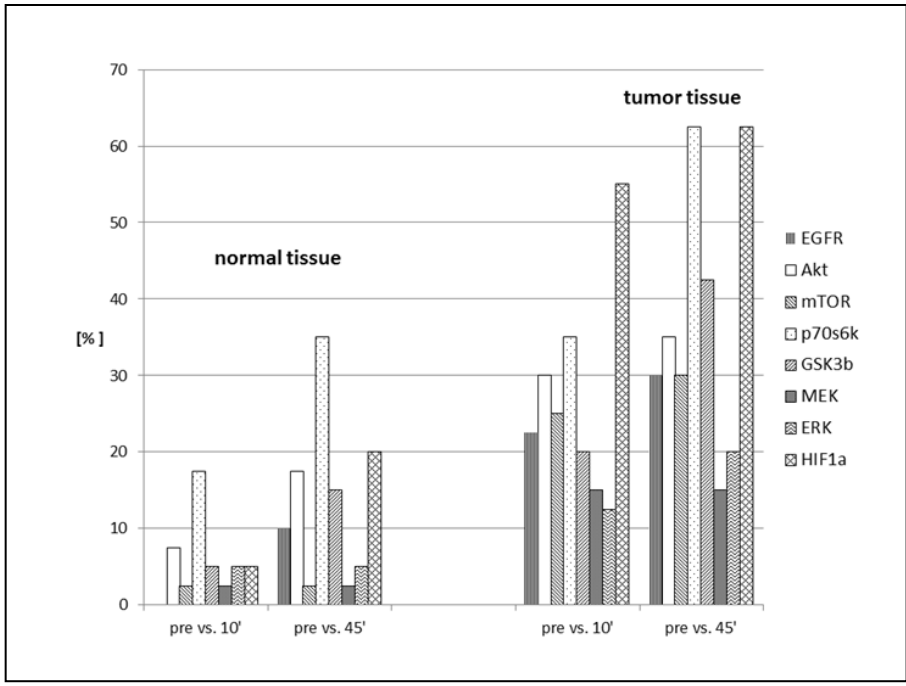
Hartmut Juhl, Indivumed GmbH, BRN

# Expression of >15% of Genes and Up to 60% of Selected Proteins Change >2-fold during Surgery and Postsurgical Processing Time

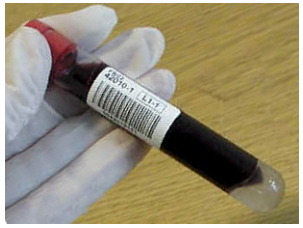
Gene Expression
Pre vs. Post Surgery




Protein Expression
Pre vs. Post Surgery



# Blood Collection and Plasma Processing: Biomarkers and Circulating Tumor Cells



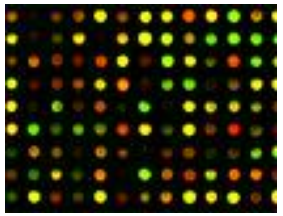
Collection Tubes and Order of draw

Processing Procedure, Temperature and Time

Distribution & Storage

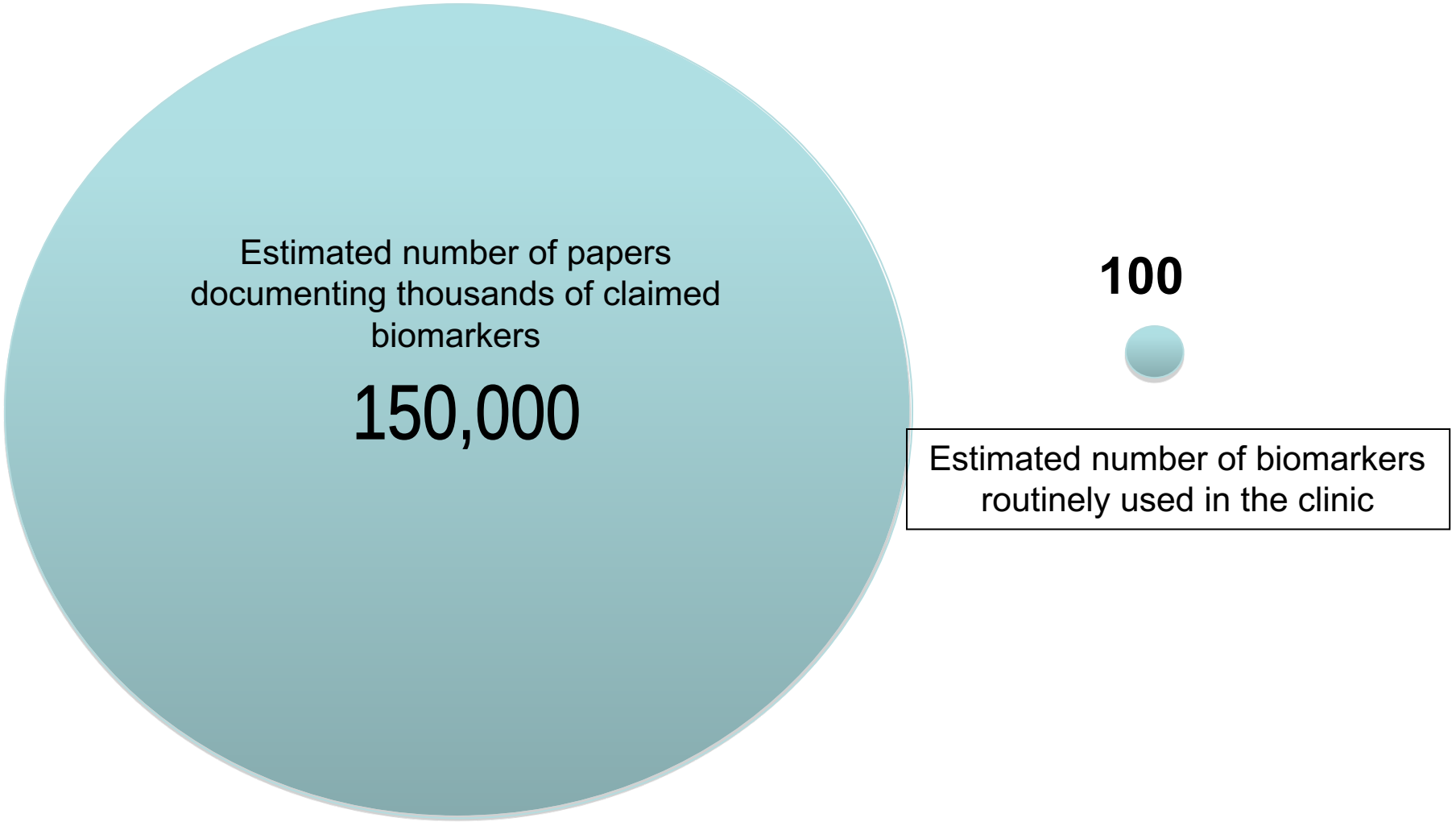
Blood Draw Procedure

Patient Consent and Preparation



Molecular Analysis

# The National Biomarker Development Alliance: Biomarker Development Fails to Deliver




Source: Poste G. Nature 469, 156-157 13 Jan 2011

# Biomarker Development Is a Team Sport

# NBDA: Understanding the Issues in Biomarker Development and Building Solutions

# The Process of Biomarker Development Is Siloed, Fragmented, and Lacks Cross-cutting Standards

Early Discovery
(Biology Verified Patient Samples)

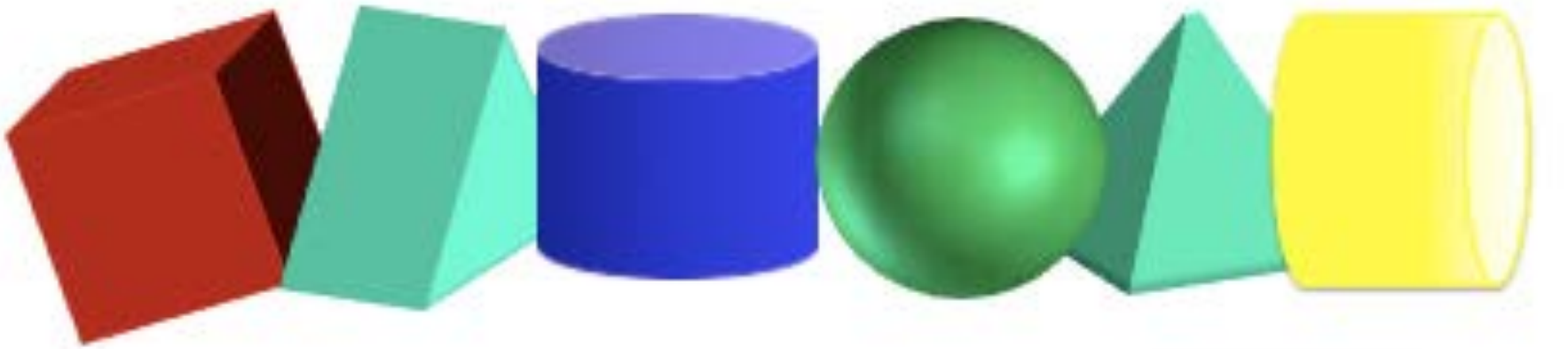
Translatable Discovery
(Clinical Measure Established)

Assay Development
(Analyte - Reagents-Technology – Robust)

Assay Performance
(Analytical Validation)

Biomarker Qualification
("Fit for Clinical Purpose)

Biomarker Validation
(Clinical Validation)



Academia

Industry

Physicians

Patients

Regulators

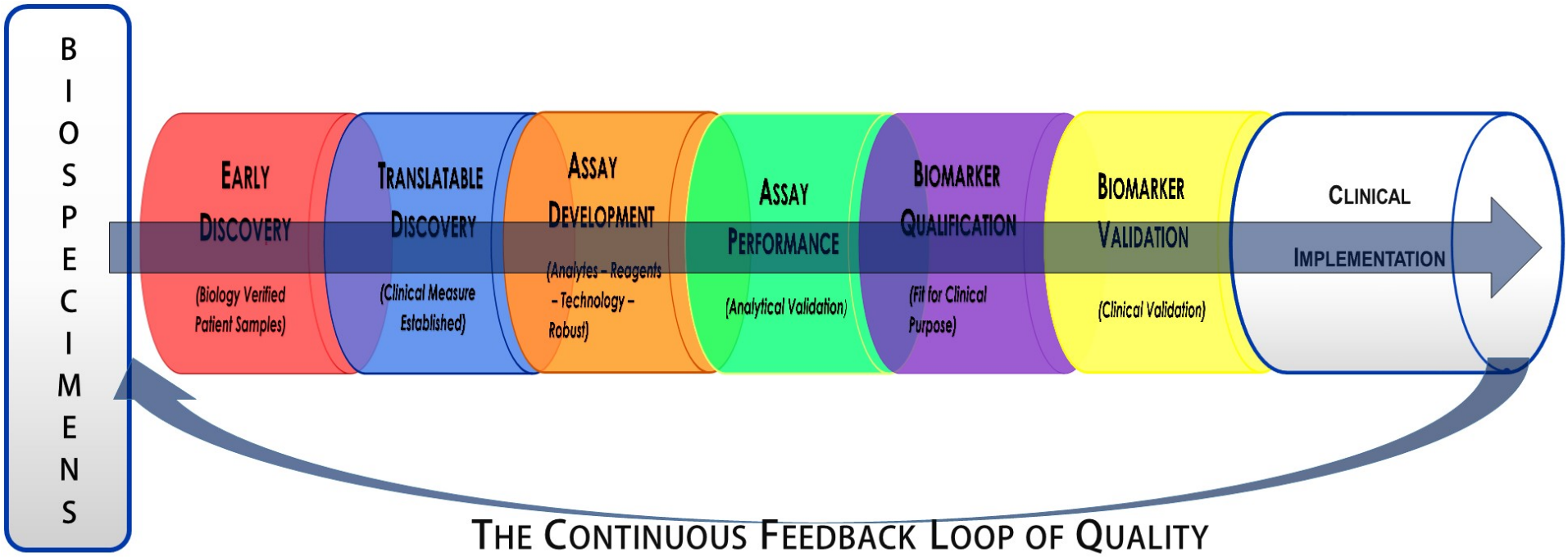
Funding Agencies

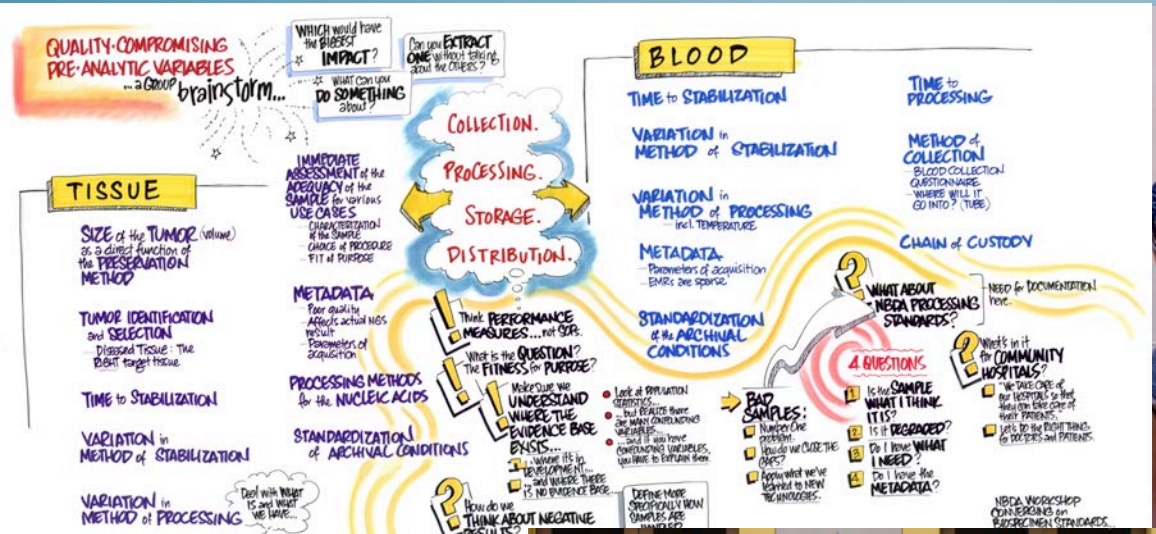
Professional Bodies

# Pervasive Barriers in Biomarker Development Identified through NBDA Think Tanks

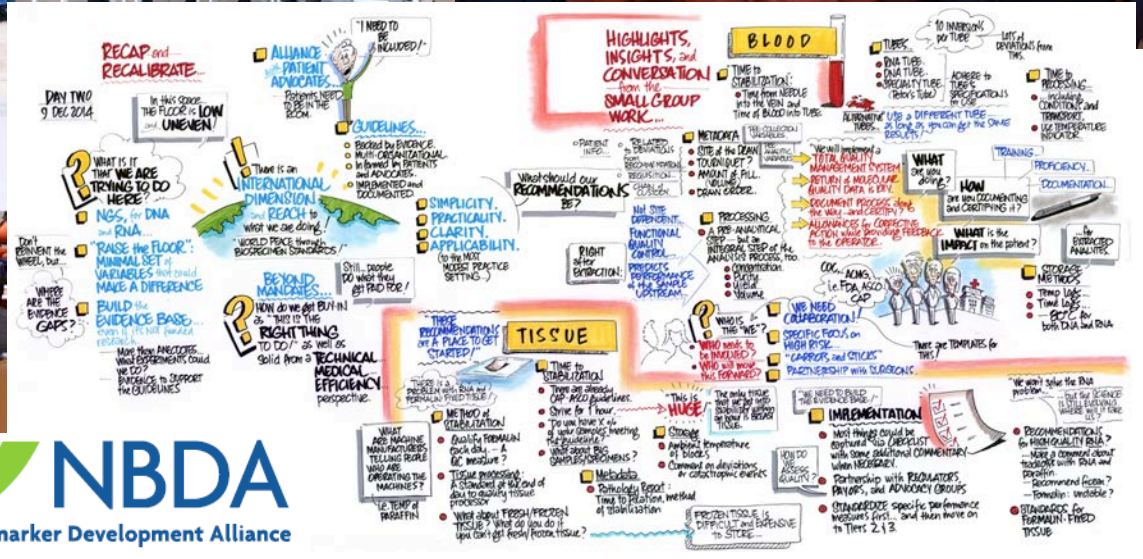
- **Poor access to rigorously annotated, fit-for-purpose biospecimens from stringently phenotyped sources**
- **Insufficient control of pre-analytical parameters**
- **Low reproducibility of academic publications**
- **Incomplete understanding of biology (normal, disease, treatment)**
- **Variable analytical standards**
- **Idiosyncratic “lab-specific” analytical methods**
- **Small studies lacking statistical power**
- **Chaotic data reporting formats and poor database interoperability**
- **Poor compliance on reporting standards by journals**
- **Poor or non-existent quality management systems**

# Biospecimens Flank End-To- End Biomarker Development










NBDA
National Biomarker Development Alliance

# NBDA Convergence Conference: The Top 10 List

Goal:

- Converge (agree) on the pre-analytical steps in the biospecimen lifecycle that MOST compromise the quality of tissue and blood for cutting edge molecular analysis: NGS and proteomics
- Identify where the greatest value can be delivered in the control of pre-analytical variation (*biggest quality bang for the buck*)

# Defining a Benchmark for Patient Biospecimens


**Pareto Principle (20/80 rule)**

**For many events 80% of the effects come from 20% of the causes**

# The TOP 10

## Tissue

1. Time to stabilization
   - Cold ischemia time
2. Method of processing
   - Section thickness
   - Mass/volume ratio
   - Temperature
3. Method of stabilization
   - Type of fixative
   - Time in fixative
4. Tissue processor variables
   - Quality of processing fluids
   - Paraffin type
   - Paraffin temperature
5. Storage conditions
6. [Metadata to be collected]

## Blood/Serum

1. Time to processing
2. Method of acquisition
   - Tube type
   - Draw order
   - Volume of tube fill
3. Method of stabilization
   - Tube inversions
4. Method of processing
   - Centrifugation speed/time
   - Temperature
5. Storage conditions
   - Freeze/thaw cycles
6. [Metadata to be collected]

# The CAP Pre-analytics for Precision Medicine Project Team

genomeweb

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## Pathologists' Group Takes Aim at Improving MDx Through Preanalytical Sample Quality

Oct 06, 2016 | Leo O'Connor

*Premium*

NEW YORK (GenomeWeb) – A group of pathologists at the annual College of American Pathologists meeting last week in Las Vegas, Nevada, said the work that they are doing to raise recognition of the need for higher quality patient samples could provide significant benefits for patient care, and lead to more reliable molecular test outcomes.

The pathologists stressed the importance to the accuracy and reliability of molecular testing of developing and following standard practice guidelines and recommendations related to the provision of higher quality preanalytical patient samples.

Preanalytics are the key to the molecular quality of specimens, which in turn determines the data quality of molecular analysis, said Carolyn Compton, chief medical officer of the National Biomarker Development Alliance in Scottsdale, Arizona, during a presentation at CAP16. Little attention is paid to controlling factors that affect patient sample quality before molecular testing is done, she added.

# CAP Validated Practicable Benchmarks: Tissue

1. Time to stabilization: 60 minutes. or less
2. Method of processing
   - Section thickness: ≤5 mm
   - Mass/volume ratio: ≥4:1, optimal ≥10:1
   - Transport temperature: ambient
3. Method of stabilization
   - Type of fixative: 10% neutral phosphate-buffered formalin
   - Time in fixative: 6-24 hours (includes time in formalin in processor)
4. Tissue processor variables
   - Maintenance schedule: Manufacturer's recommendation or a validated deviation
   - Paraffin type: low melt <60°C
   - Total time in processor: 7.5-8 hours (forbid non-standard practices: e.g., "topping off with non-standard solutions)
5. Storage conditions: Ambient (e.g., 20-25° C)
6. [Metadata to be collected]: Any deviation from the above recommendations

# Envisioned Result

Historic transformation of practice with far-reaching impact:

•Variably variable and unknown quality ➔ uniform, known quality that is consistent with molecular analysis

•Simultaneous impact on both clinical and research results

•“Convenience samples” become fit for purpose!

•A “bar” is established that may be electively raised as needed to meet requirements of specific analysis types/platforms

- There will, at last, BE a bar to raise
- It’s about time

# The Challenge for Pathology

Garbage in…

…Garbage out

# Preanalytical Processing: The Biospecimen Quality Imperative

Carolyn Compton, MD, PhD
Chair, Scientific Advisory Committee, Indivumed GmbH
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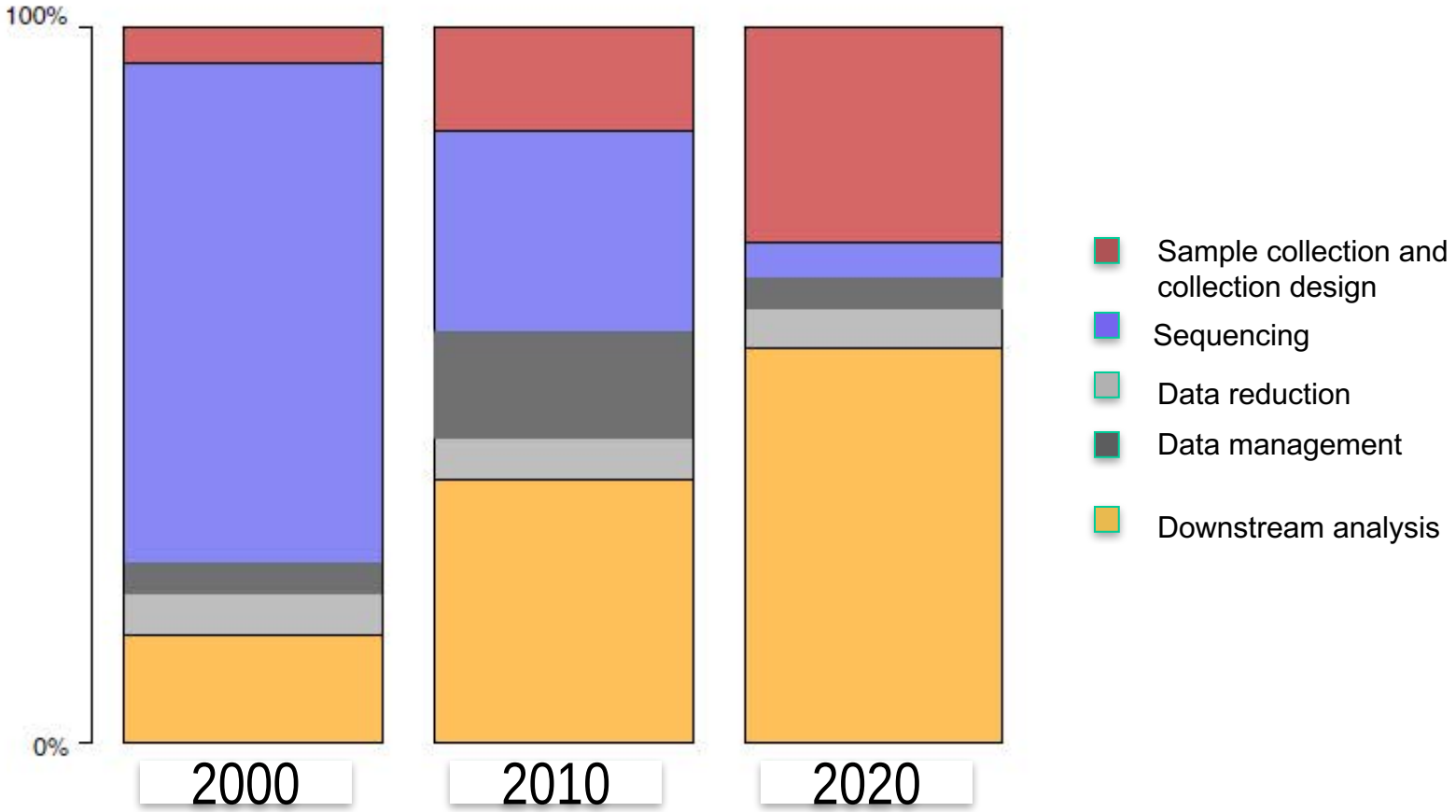
# Estimating the Changing Aspects of NGS

## Are pathologists prepared for what's coming?




From Ken Bloom, MD, GE Healthcare, June 2014

# Biomarkers and the Laboratory

**Biomarker:** **A *measurable* characteristic used as an indicator of a biological state or condition**

**Often a protein or a set of proteins measured in cells, tissue, blood but may be any class of biomolecule – DNA, RNA, miRNA, other**

- **Early detection, surveillance**
- **Prognosis, prediction**
- **Choice of treatment**
- **Monitoring of treatment**
- **Monitoring of disease**
- **Drug and diagnostics development – clinical trials; patient selection, efficacy, toxicity, surrogate endpoints**

# Getting to Precision Medicine: Biomarkers Are the Driving Force

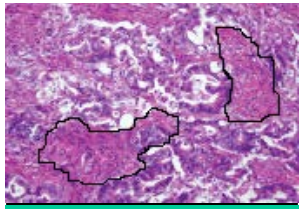
**Vision of 21st Century Medicine: Greater Efficiency and Efficacy**

- **Better understanding of the biology of disease**
- **Diagnosis based on molecular characterization of disease**
- **Rational treatment using molecularly targeted agents**
- **Connection of research and clinical practice in seamless feedback loop**

↑

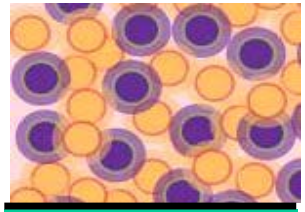
**ALL OF THESE ARE BIOMARKER-DRIVEN**

# The Right Answers Depend on the Right Stuff



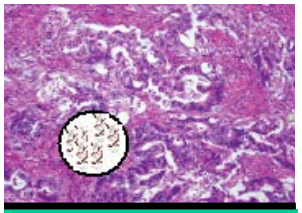
**% Tumor content**

Tumor cells are typically mixed with normal tissue. Tumor content may be enriched by macro-dissection



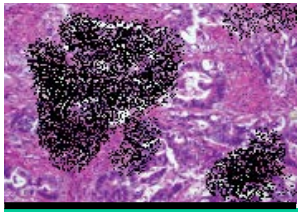
**% Mutant copies**

Tumors have background of wild-type DNA. Challenge to detect low % mutant alleles



**% Amplifiable DNA**

Tissue fixation damages DNA. Necrotic cells may not have amplifiable DNA



**% Fixation inhibitors**

Natural and introduced inhibitors may interfere with amplification

FFPET = formalin-fixed, paraffin-embedded tissue