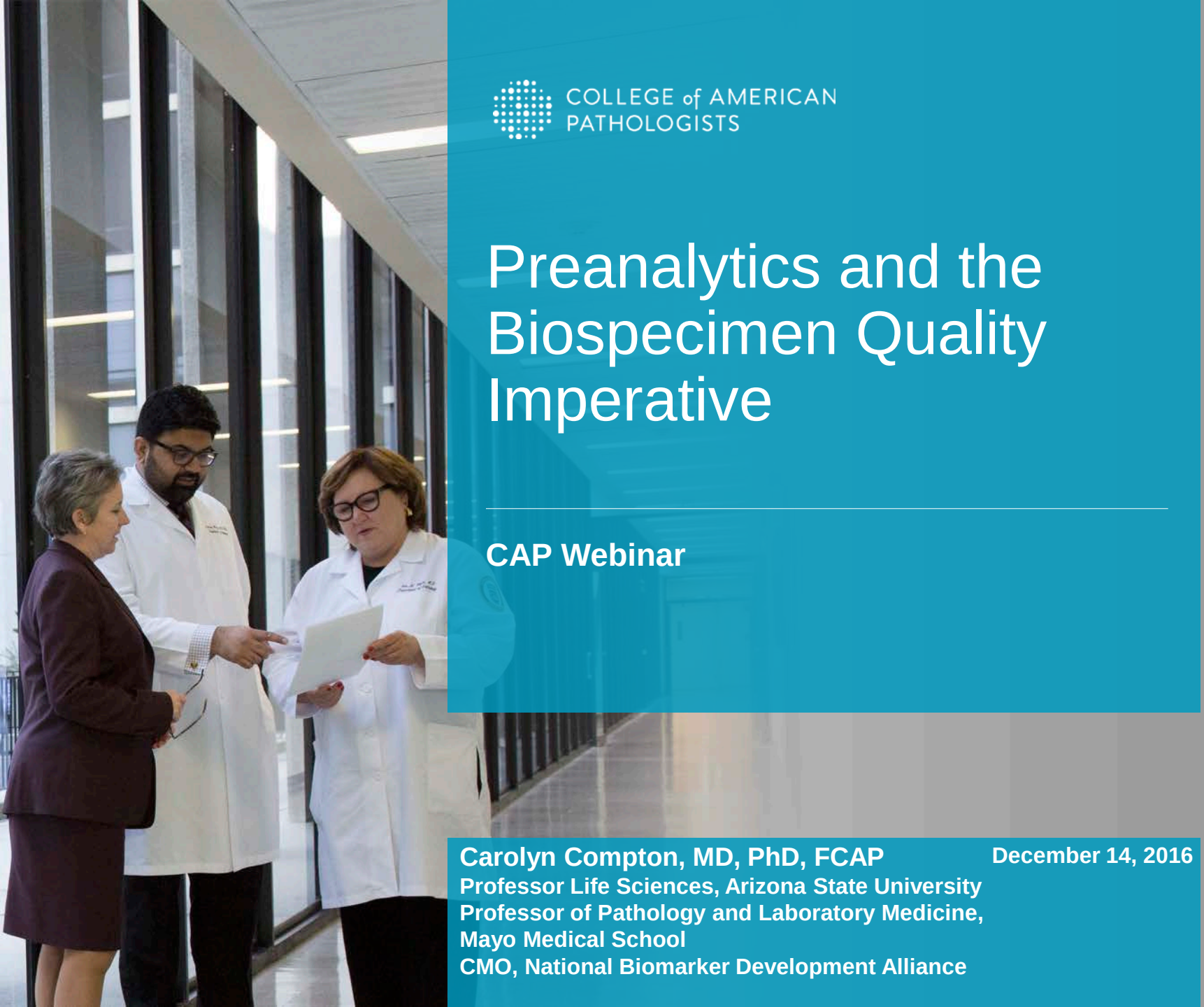

COLLEGE of AMERICAN PATHOLOGISTS

Preanalytics and the Biospecimen Quality Imperative

CAP Webinar

Carolyn Compton, MD, PhD, FCAP
Professor Life Sciences, Arizona State University
Professor of Pathology and Laboratory Medicine, Mayo Medical School
CMO, National Biomarker Development Alliance

December 14, 2016

Webinar Host

- **This series is sponsored by the Personalized Healthcare (PHC) Committee**
- **Today's webinar host is Allison M. Cushman-Vokoun, MD, PhD, FCAP**

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Housekeeping

- **This presentation will be recorded. The recording and PDF will go out to all registrants in several weeks**
- **All lines are muted during the presentation**
- **Please send in your questions as you think of them via the “Question box” in your control panel**

CAP

Carolyn Compton, MD, PhD, FCAP

- **Academic pathologist specializing in gastrointestinal disease and is board certified in both anatomic and clinical pathology**
- **Professor Life Sciences, Arizona State University**
- **Professor of Pathology and Laboratory Medicine, Mayo Medical School**
- **CMO, National Biomarkers Development Alliance and Complex Adaptive Systems Institute**

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Disclosures

- **Consultant –** Indivumed, CloudLims
- **Advisory Board** - BBMRI Scientific and Ethical, and Roche Global Pre-Analytics
- **Board of Directors** - HealthTell
- **Executive Committee** – AJCC
- **Member -** US Technical Advisory Group to ISO (Biobanking and Biotechnology) and CLSI MM13 Development Committee

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Disclaimer, continued

- **Opinions expressed by the speaker are the speaker's own and do not necessarily reflect an endorsement by the CAP of any organizations, equipment, reagents, materials, or services used by participating laboratories.**

Presentation Objectives

- **Objective 1:** **Demonstrate the essential role of molecular biomarkers from patient biospecimens in precision medicine**
- **Objective 2:** **Explain the connection between patient biospecimen quality and reproducible molecular assay results**
- **Objective 3:** **Discuss the impact of pre-analytical variables on molecular quality and composition of patient specimens**
- **Objective 4:** **Describe the actions being undertaken by the CAP to address pre-analytical variation in every day pathology practice**

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Presentation Content

- **The Case for Pre-Analytics in Pathology Practice**
- **The CAP Personalized Healthcare Committee and the Pre-analytics for Precision Medicine Project Team**
- **Tissue, Blood, and Cytology Specimens**
- **The Plan for Implementation**
- **Expected Outcome**

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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:**
  - **Molecularly targeted therapy for disease**
  - **Molecular profiling for drug metabolism in patient**
- **Connection of research and clinical practice in seamless feedback loop**

↑

ALL OF THESE ARE BIOMARKER-DRIVEN

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Biospecimen Quality Drives Both Molecular Medicine and Translational Research

Molecu

DETERMINES QUALITY HERE

lopment

PRECISION MEDICINE

Biospecimen Analysis

Biospecimen Collection

QUALITY HERE

Biospecimen Handling and Processing

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Pre-analytics Affect the Molecular Quality and Composition of Biospecimens

Specimen is viable and biologically reactive

Molecular composition subject to further degradation post-stabilization

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

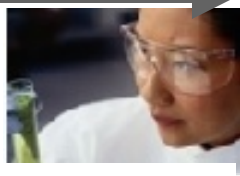

Acquisition


Handling/ Processing


Storage


Distribution


Scientific Analysis


Restocking Unused Sample

Pre-acquisition

Post-acquisition

Pre-analytical

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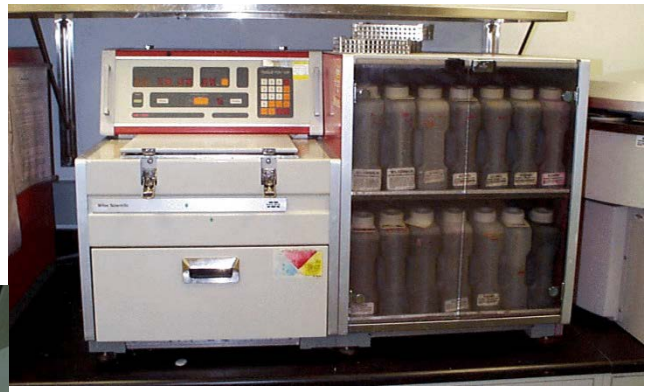
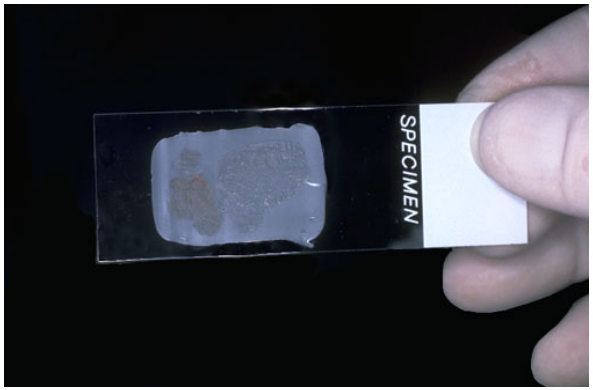
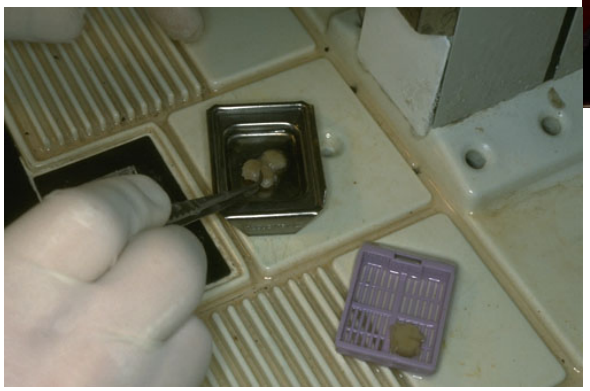
Pre-Analytic Variables May Comprise Test Results

- **Processing Methods**
  - **Fixation time, type and method**
  - **Processor fluid maintenance**
  - **Paraffin temperatures**
- **Specimen transport and storage**
  - **Temperature, duration, dehydration, desiccation, oxidation**







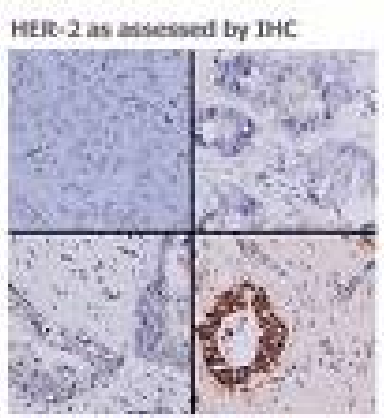
Biospecimen Quality Impacts Both Clinical and Research Outcomes

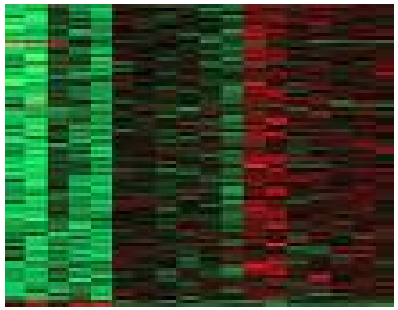
Effects on Clinical Outcomes

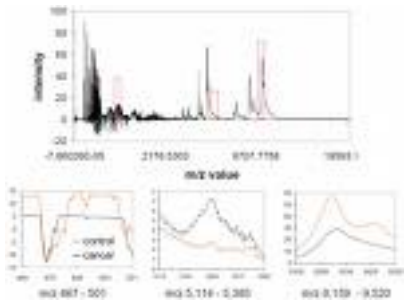
- Potential for incorrect diagnosis
  - Morphological/immunostaining artifact
  - Skewed clinical chemistry results
- Potential for incorrect treatment
  - Therapy linked to a molecular test on a biospecimen (eg, HER2 in breast cancer)

Effects on Research Outcomes

- Irreproducible results
  - Variations in gene expression data
  - Variations in post-translational modification data
- Misinterpretation of artifacts as biomarkers







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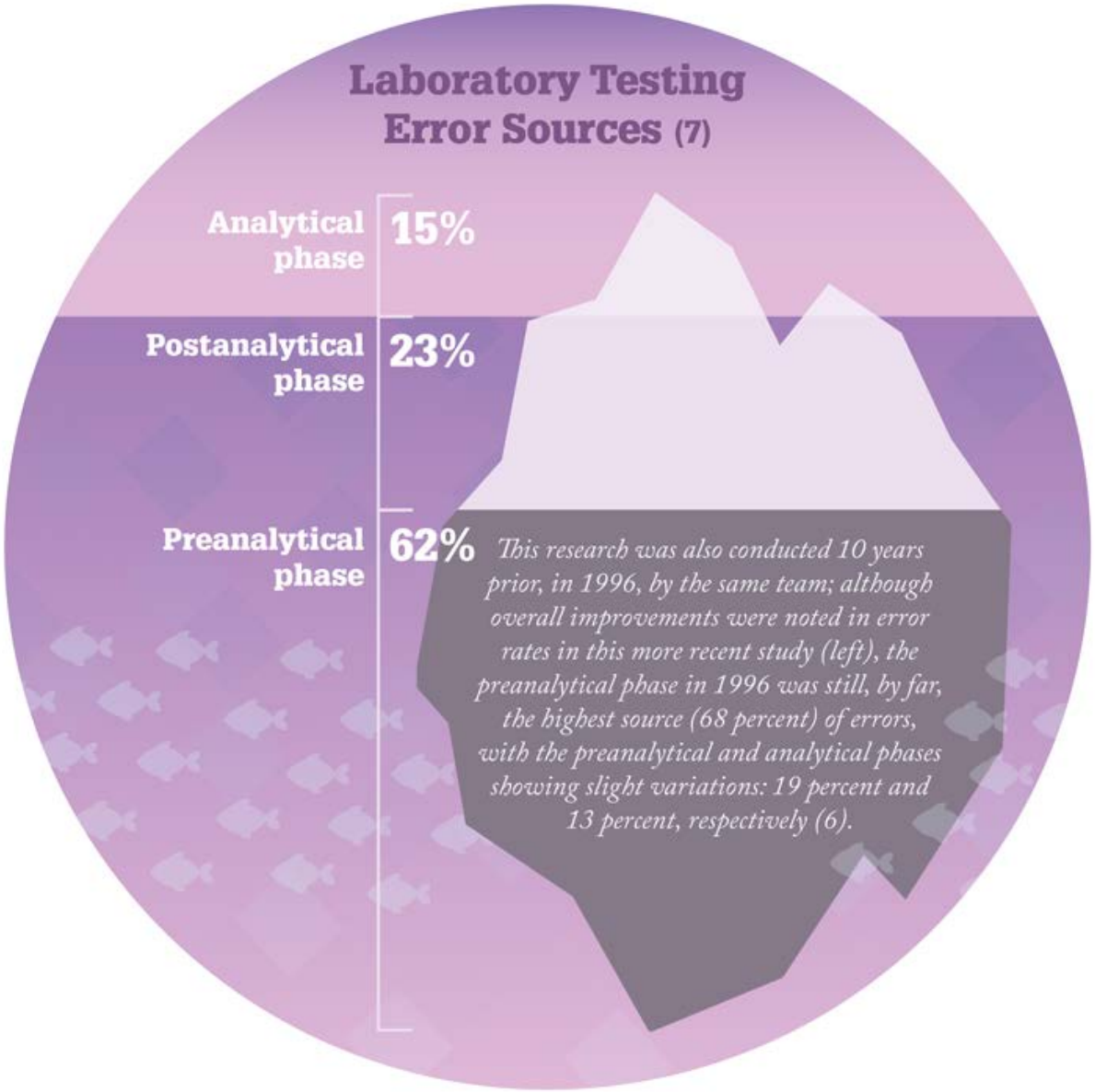

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Simundic AM. The Pathologist, issue 0515

Molecular Changes are Real and Can Alter Biomarker Levels

- **Under and over-expression of specific genes has been associated with certain diseases like cancer**
- **Similar expression changes have been noted in response to environmental changes and biologic stresses**
  - **Temperature**
  - **pH**
  - **Nutrient availability**
  - **Oxygen deprivation**

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Molecular Changes are Real and Can Alter Biomarker Levels

- **Gene changes can occur within 15 minutes**
- **Post-translational changes such as methylation or phosphorylation can occur in seconds.**
- **DETERMINING WHICH CHANGES ARE DISEASE-RELATED AND WHICH ARE ARTIFACT IS ESSENTIAL AND CAN BE DIFFICULT**

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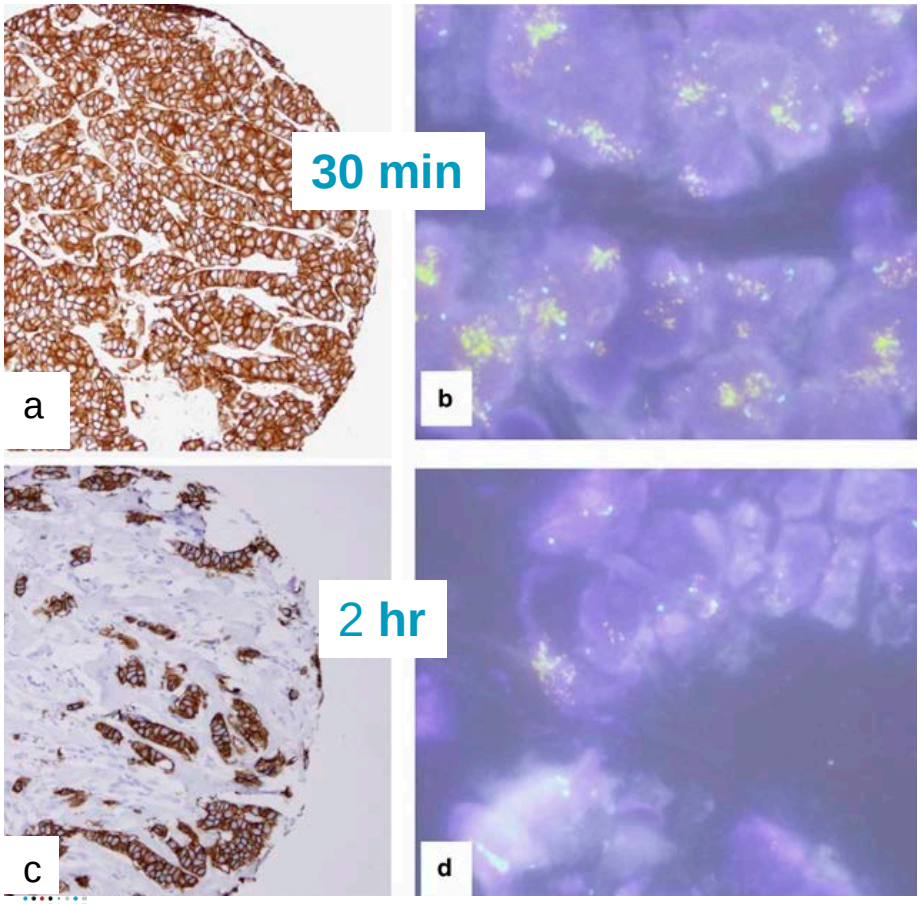
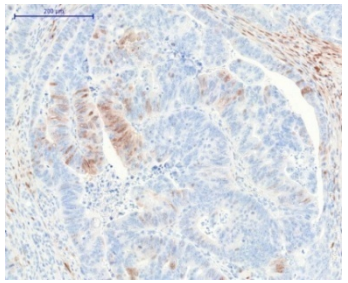
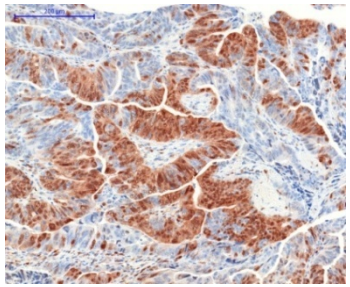
Cold Ischemia and Molecular Assay Results

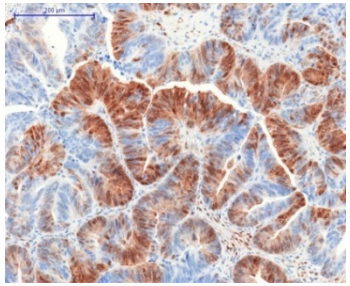
HER2 IHC and FISH in Breast Cancer: Loss of Biomarker Signal with Time to Fixation



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







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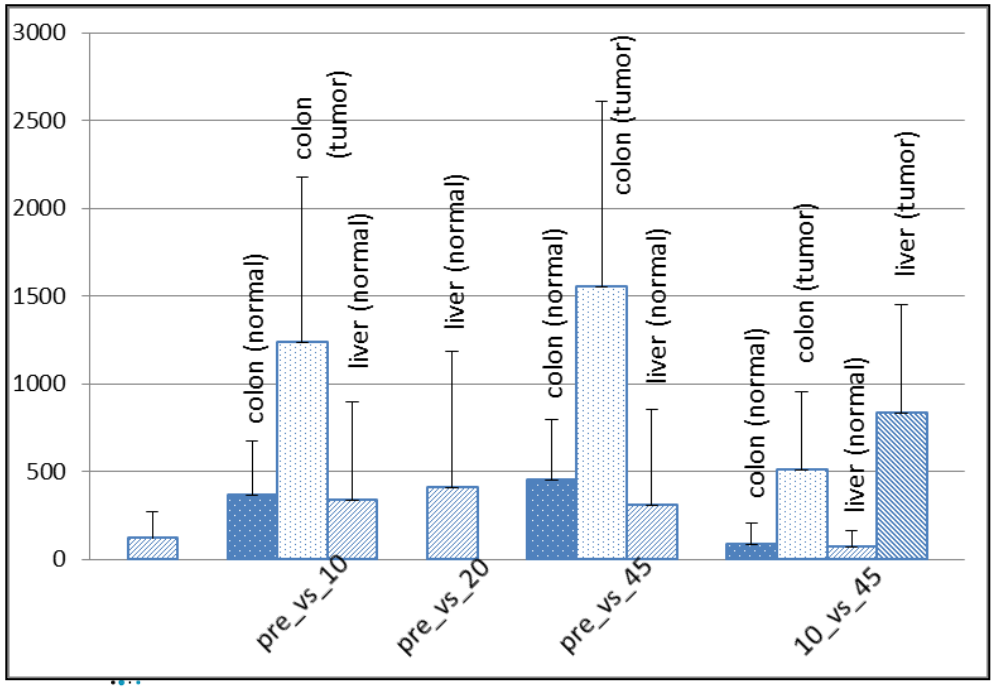
Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

Hartmut Juhl, Indivumed GmbH, BRN

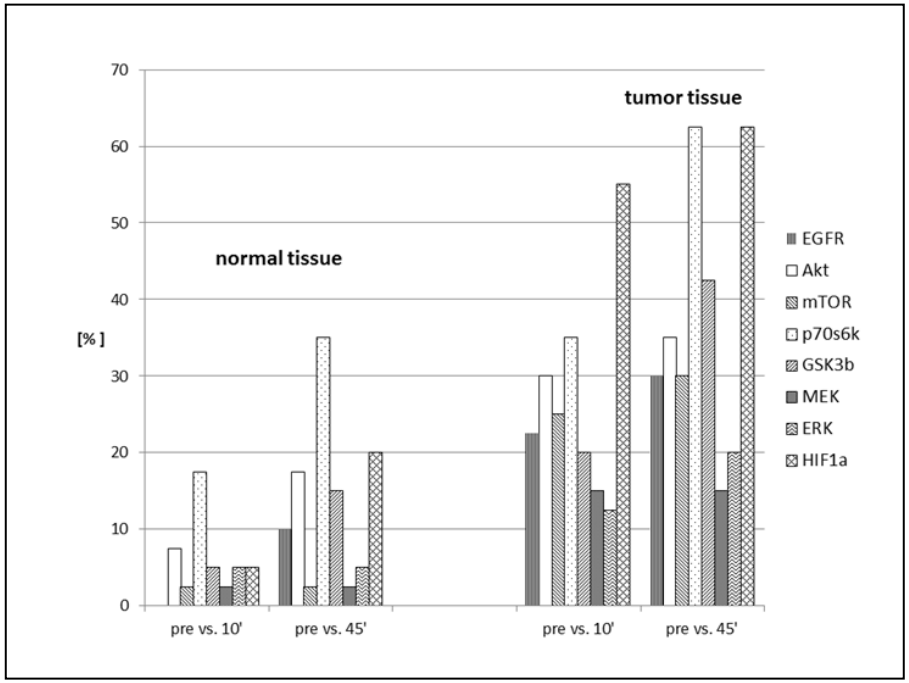
Pre-Analytics Alter Transcription and Translation

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



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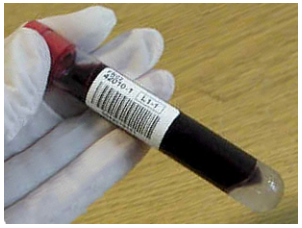
Blood Collection and Plasma Processing: Biomarkers, cfDNA and Circulating Tumor Cells



Collection Tubes and Order of draw

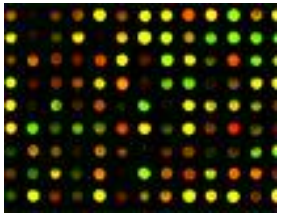
Processing Procedure, Temperature and Time

Blood Draw Procedure

Distribution & Storage

Patient Consent and Preparation

Molecular Analysis



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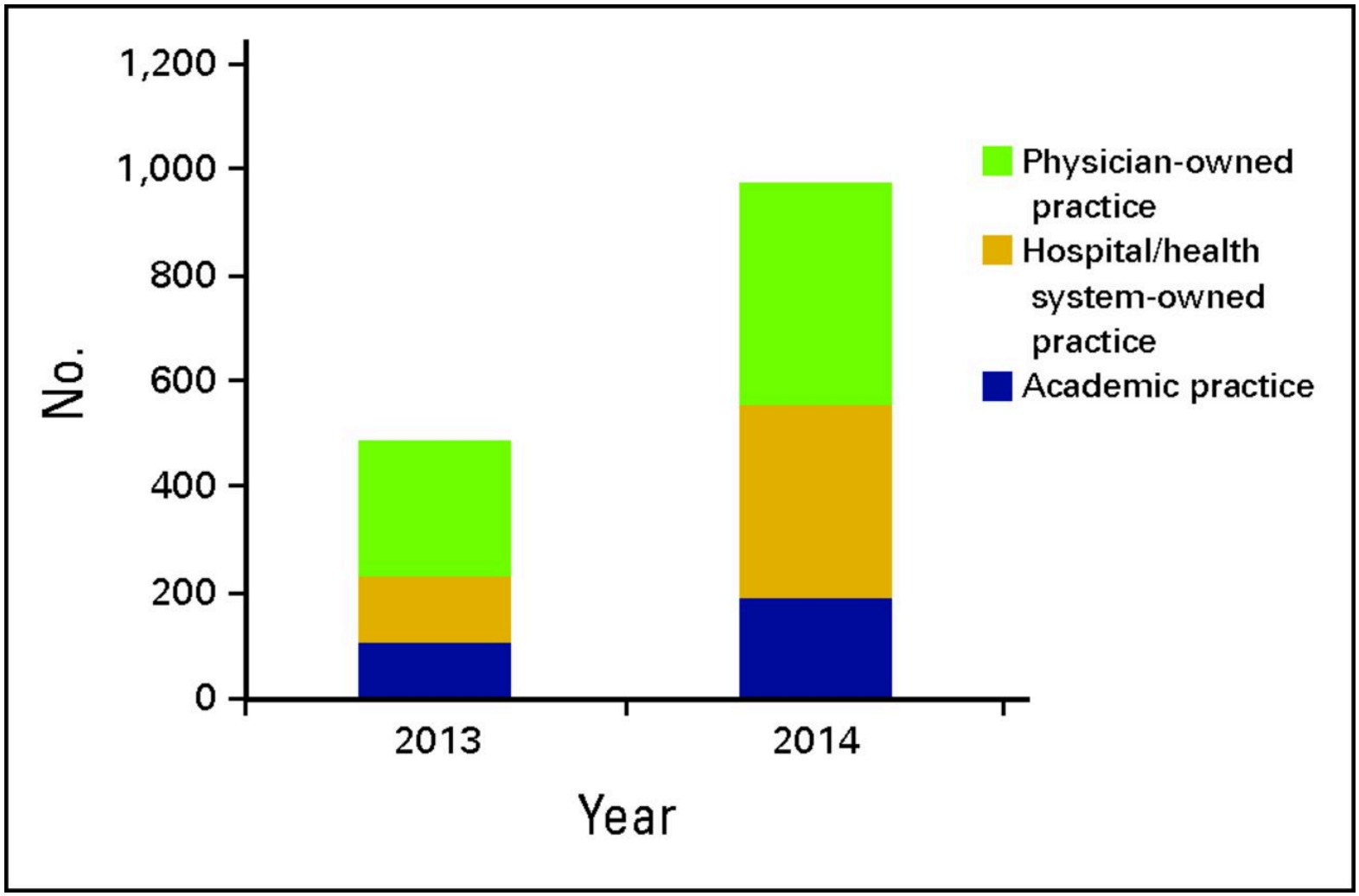
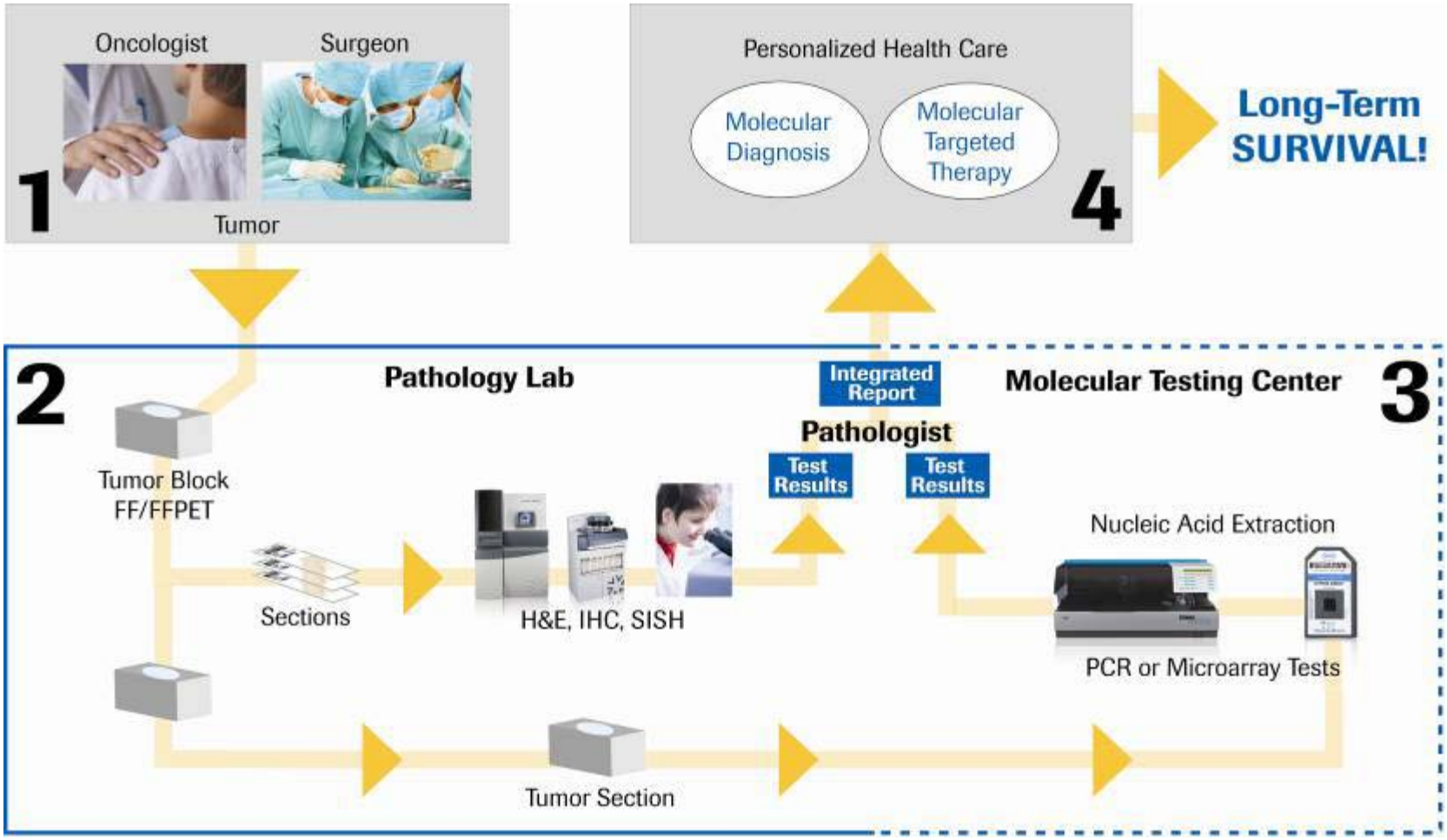
Most Cancer Diagnosis Occurs in the Community



the American Society of Clinical Oncology JOP
doi:10.1200/JOP.2015.003772

Todays Anatomic Pathology Lab



Walk E. Cancer: Critical Role of Pathology in Personalized Health Care, CAP PHC Webinar Series, 2010

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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
  - **ASCO-CAP guidelines for a single specimen type**

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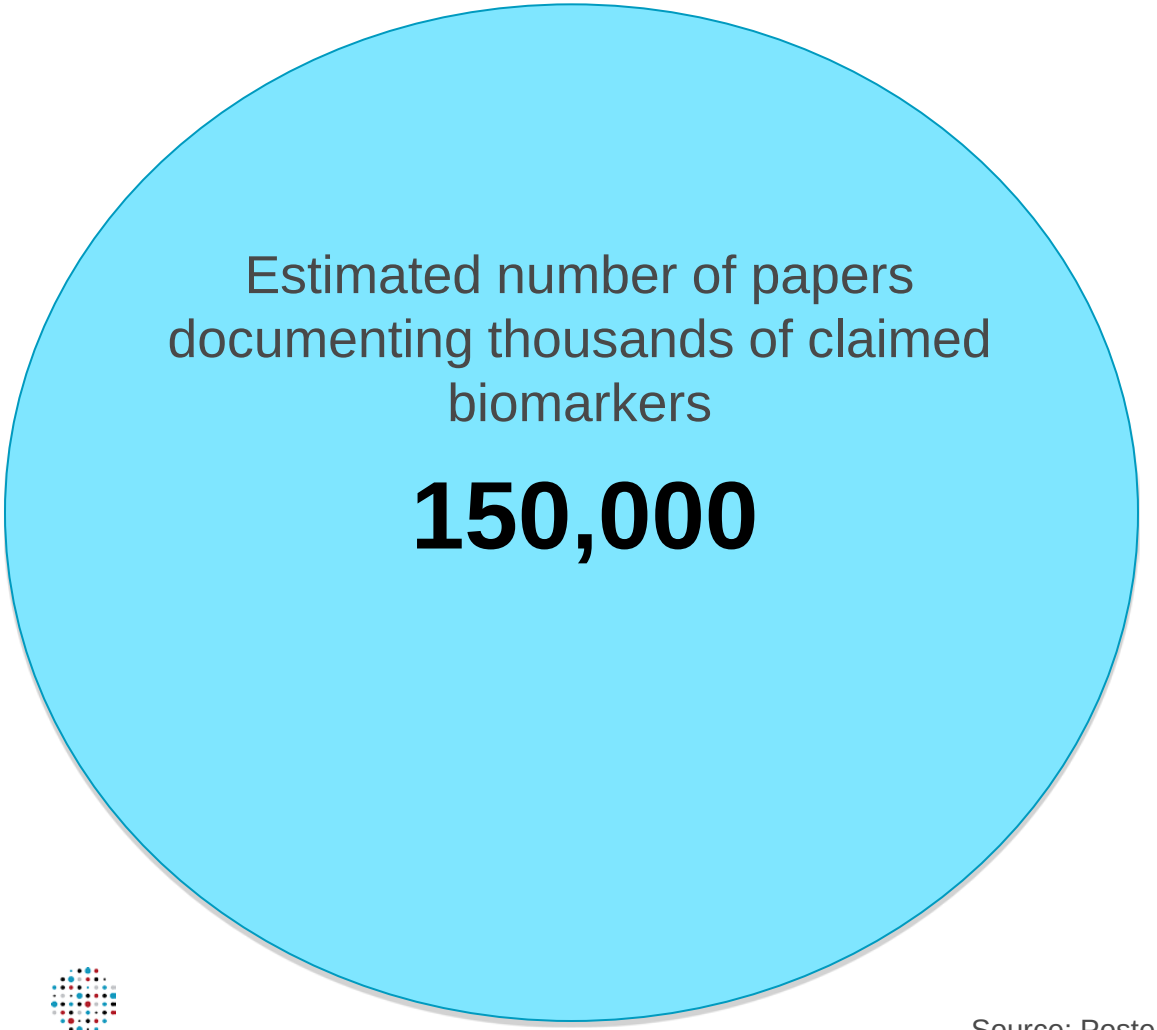
Biomarkers Needed:
Many Are Reported, Few Are Qualified



100

Estimated number of biomarkers routinely used in the clinic

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

The IOM 2016: Recognition of the Urgency

- **This year, the Institute of Medicine released a report entitled *Biomarker Tests for Molecularly Targeted Therapies: Key to Unlocking Precision Medicine***
- ***Ten goals for the nation are* goals are put forth**
- **Goal #9 addresses biospecimen quality:**
  - **9) Enhance specimen handling and documentation to ensure patient safety and the accuracy of biomarker test results.**
  - **The reliability of biomarker test results depends on the quality of the patient specimens. Professional organizations and health care institutions should develop and implement standards for obtaining adequate specimens.**

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Pervasive Barriers to Biomarker Development

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

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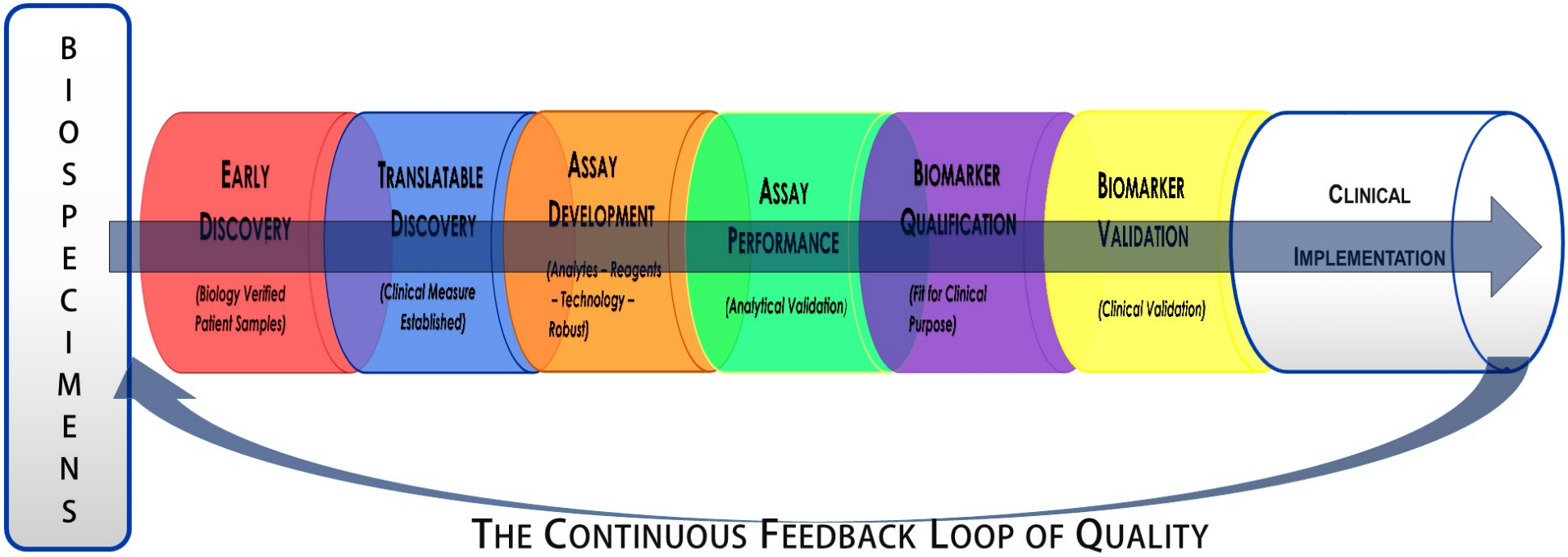
Patient Specimens Drive the Process - Continuous Loop



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National Biomarker Development Alliance

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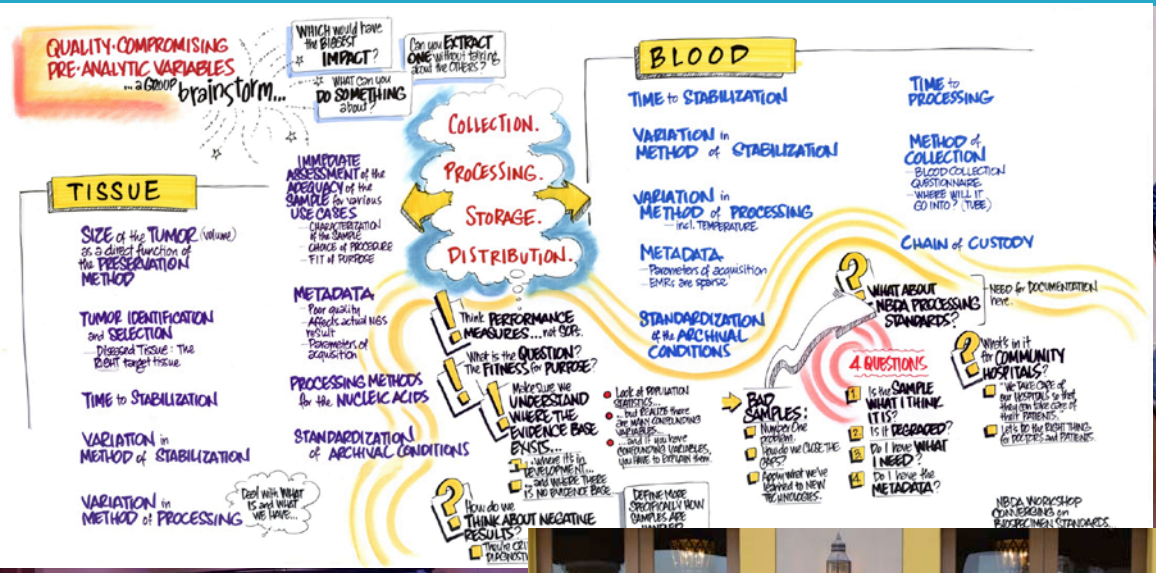

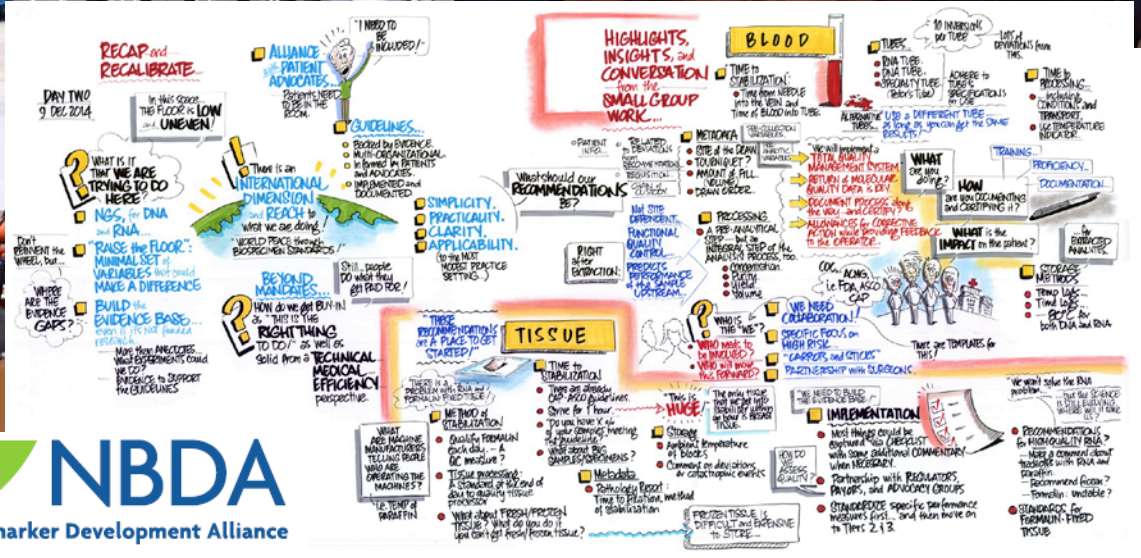

NBDA
National Biomarker Development Alliance

NBDA Convergence Conference Summit

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**
  - **“Top 10 List”**
- **Identify where the greatest value can be delivered in the control of pre-analytical variation (*biggest quality bang for the buck*)**
  - **“Top 5 List”**

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Defining a Benchmark for Patient Biospecimens

Pareto Principle (20/80 rule)

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

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Top 5 Lists

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]

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Pre-Analytics for Precision Medicine Project Team: Objectives

- **Objective 1: Vet the TOP 5 lists**
- **Objective 2: Establish performance metrics around the Top 5 that are:**
  - **DATA-DRIVEN**
  - **PRACTICABLE**
- **Objective 3: Educate pathologists and pathology workforce (pathology assistants) through the CAP, AAPA and Surgeons through the Commission on Cancer of the ACS about pre-analytics**
- **Objective 4: Implement performance metrics through the CAP Laboratory Accreditation Program checklists**

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Process to Obtain Draft Recommendations

- **2013-2015 literature search for tissue and blood pre-analytics**
- **Additional literature search for cytology pre-analytics**
- **Create and vet data collection form for reviewed abstracts**
- **Two team members review abstracts of each potential article to identify articles potentially relevant data for pre-analytics**
- **Narrow to pre-analytics relevant for routine clinical practice**
- **Create and vet data collection form for full manuscript reviews**
- **For tissue and blood: enter data into extract form for each full article reviewed and compare to existing authoritative guidelines for tissue and blood**

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Benchmarks from Authoritative Sources

- **College of American Pathologists – American Society of Clinical Oncology (CAP-ASCO)**
- **International Standards Organization (ISO TC276)**
- **Clinical and Laboratory Standards Institute (CLSI)**
- **International Society for Biological and Environmental Resources (ISBER)**
- ***European Committee for Standardization (CEN)***
- ***National Cancer Institute (NCI)***

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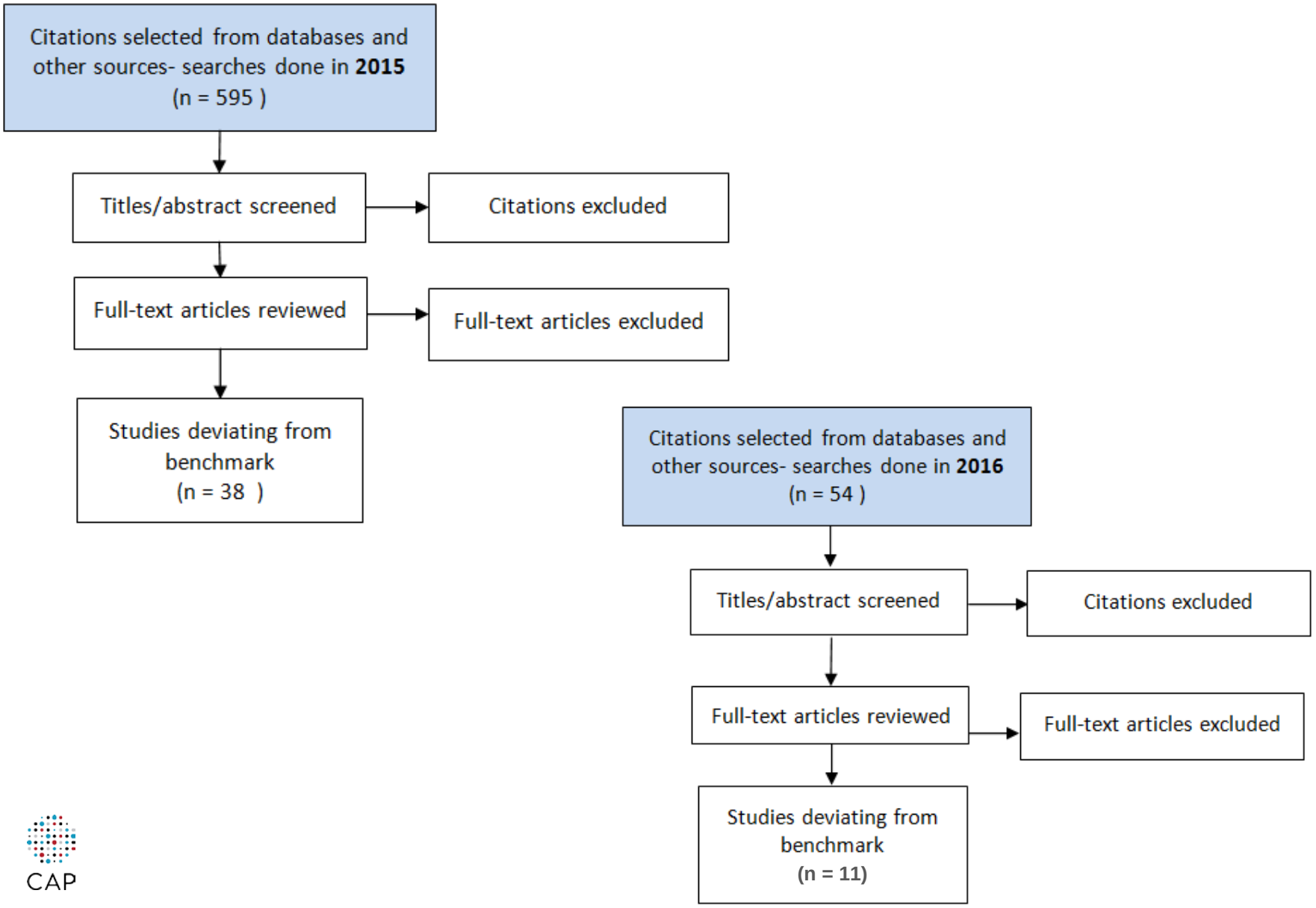
Pre-Analytics for Precision Medicine



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PPMPT Proposed 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)

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PPMPT Proposed Benchmarks: Tissue

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: eg, "topping off with non-standard solutions)
5. **Storage conditions**
   - Ambient (eg, 20-25°C)
6. **[Metadata to be collected]**
   - Any deviation from the above recommendations

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PPMPT Proposed Benchmarks: Blood/Serum

1. **Time to first processing step**
   - **<60 minutes**
2. **Specimen acquisition**
   - **Tube type: (specialized for a specific molecule species vs. not)**
     - **If processing time is to be >2-3 hours, use acid-citrate-dextrose (ACD) tube**
     - **EDTA for proteomics studies**
     - **Do not use lithium heparin for nucleic acid amplification studies**
     - **Sodium citrate for coagulation studies**
   - **Volume of tube fill:**
     - **Manufacturer's recommendation**
     - **If less than specified amount for tubes with additives, document variance.**
   - **Draw order:**
     - **Culture bottles**
     - **light blue (citrate)**
     - **gold (gel, serum)**
     - **red (no gel, serum)**
     - **green or tan (heparin)**
     - **lavender or tan (EDTA)**
     - **royal blue (EDTA),**
     - **gray (sodium fluoride)**
     - **tubes with other additives (eg, yellow – acid-citrate-dextrose (ACD).**

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PPMPT Proposed Benchmarks: Blood/Serum

3. **Method of stabilization**
   - **Tube inversions: Manufacturer's recommendations**
4. **Method of processing**
   - **Centrifugation speed/time: Variable depending upon validated protocol and biomolecule being studied.**
   - **Temperature: Ambient unless validated protocol dictates otherwise**
5. **Storage conditions**
   - **Freeze-thaw cycles: Nucleic acids and proteins ≤ 1 (use aliquots)**
6. **[Metadata to be collected]**
   - **Any deviation from the above recommendations.**

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Project Team, Liaison Members, and CAP Staff

PHC Committee Members:

- **Carolyn Compton, MD, PhD – Project Team Chair**
- **Sophia Yohe, MD**
- **Ken Bloom, MD**
- **Allison Cushman-Vokoun, MD, PhD**
- **Jordan Laser, MD**
- **Jan Nowak, MD, PhD**
- **Jessica Crothers, MD**
- **Matt Anderson, MD, PhD**
- **Michael Misialek, MD**
- **Anna Berry, MD**
- **Andrew Schade, MD, PhD**

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Project Team, Liaison Members, and CAP Staff

Liaisons:

- **Informatics - Jim Robb, MD**
- **Biorepository Committee - Phil Branton, MD**
- **PERT - George Birdsong, MD**
- **Cancer Committee - Joseph D Khoury MD**
- **Cytology Committee - Carrie Marshall, MD**
- **Cytology Committee - Kristen Natale, DO**

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Project Team, Liaison Members, and CAP Staff

CAP Staff:

- **Patty Vasalos, Technical Manager, Proficiency Testing; Lead Staff**
- **Molly Hansen, Technical Specialist, Proficiency Testing**
- **Jill Kaufman, PhD; Director of PHC**
- **Tony Smith, MLS-Records and Information Manager**
- **Brooke Billman, MLS-Records and Information Manager**
- **Kelly Westfall, PT Operations Specialist**

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Cytology Preparation Techniques with Reports of Successful Use for Molecular Analysis

FNAs	**Cellient Cell Block**
DQ Stained Slides	**Touch Prep**
PreservCyt Suspensions	**Frozen**
Cytolyt Suspensions	**Scraping**
Cytospin	**Saline Then Frozen**
Ethanol fixed	**Pap Stained Slides**
Traditional Cell Block	(Proprietary Suspensions)*
Unstained	(Cytorich Red Suspensions)*
Airdried Unstained Slides	(Spray Fixative)*

* No data

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Planned Deliverables 2017

- **White papers on pre-analytics for tissue and blood**
- **Launch of joint process for cytology specimens with Cytopathology Committee**
- **Team review of findings and proposed practice metrics for tissue/blood with specified CAP Scientific Committees and Council on Scientific Affairs**
- **Proposal of checklist questions referable to the TOP 5 pre-analytical issues, working with LAP Checklist Committee**
- **Submission of request to the CAP Center for guideline creation**
- **Goal: implementation in practice through the Laboratory Accreditation Program**

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Envisioned Result

Historic transformation of practice with far-reaching impact:

- **Variably variable and unknown provenance ➔ uniform, known provenance**
- **Specimen *quality* for every patient that is consistent with molecular analysis**
- **Simultaneous positive impact on both clinical and research analysis results**
- **“Convenience samples” from clinical practice 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**

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Summary

Garbage in…

…Garbage out

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Summary

- **This is for patients everywhere**
- **It’s the right thing to do**

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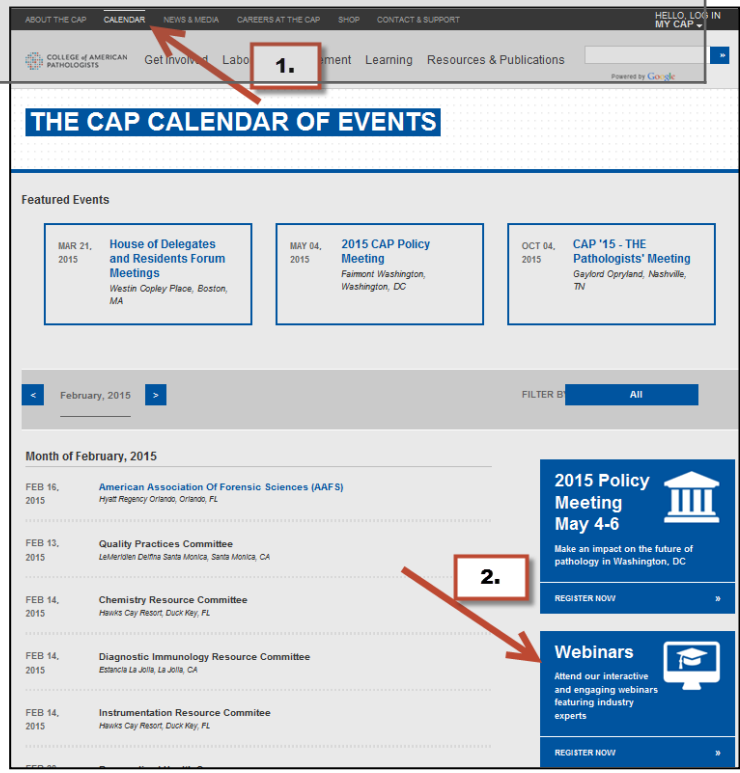
Save the Date for Upcoming Complimentary CAP PHC Webinars

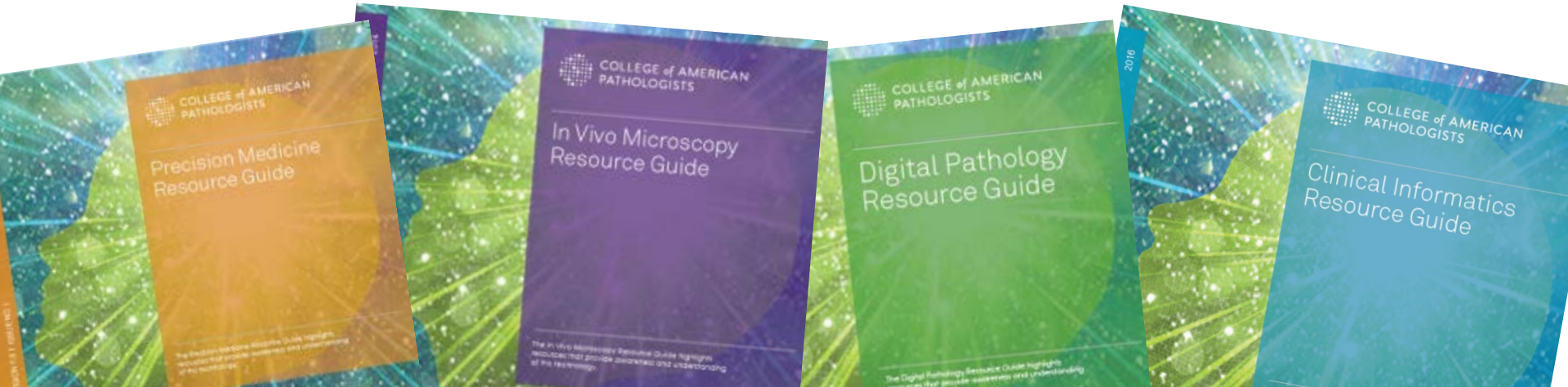
DATE	TOPIC	SPEAKERS
Jan 18, 2017 11 AM CT	HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma	Mary Kay Washington, MD, PhD, FASCP, FCAP Jaffer A. Ajani, MD, FASCO

Register for upcoming webinars:
www.CAP.org > Calendar > Webinars



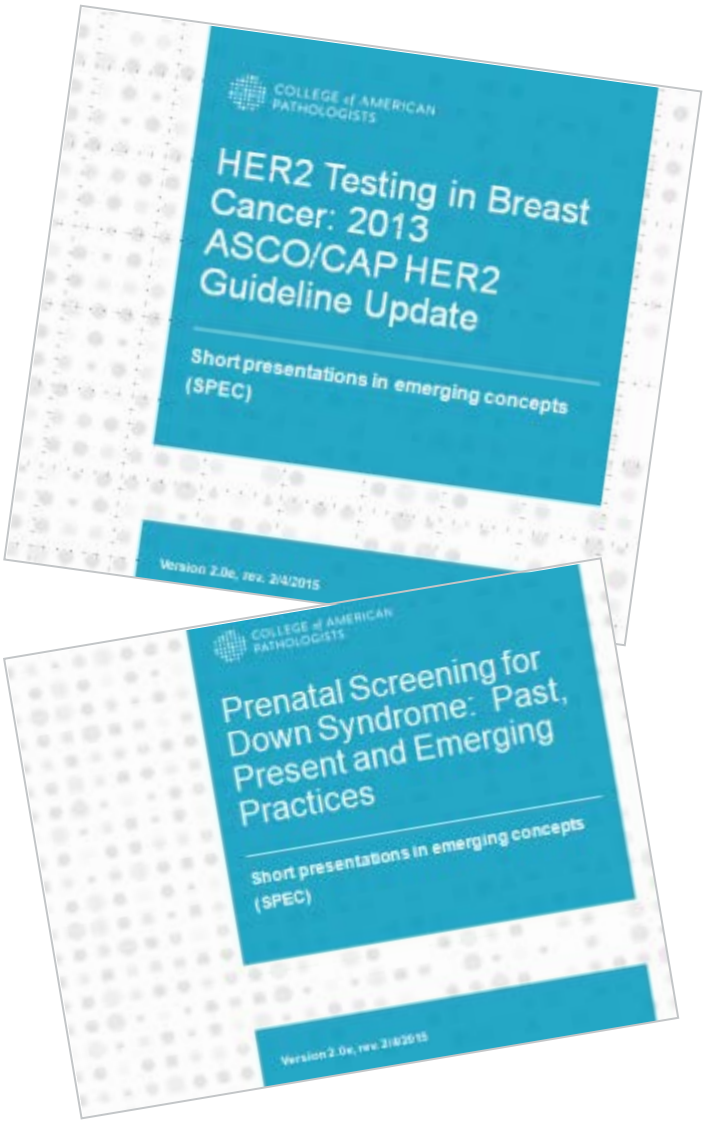
CAP's Pathology Resource Guide: Precision Medicine

- **The CAP has created the Pathology Resource Guides to assist pathologists in understanding key emerging technologies.**
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **New topics are Renal Tumors, cell free DNA (cfDNA), and PD-L1 as well as other emerging topics**
- **Access them www.cap.org > Resources and Publications**



CAP

See, Test & Treat® brings cancer screenings to women in need!

- See, Test & Treat is a CAP Foundation-funded program that brings free, same-day cervical and breast cancer screening, diagnoses and follow-up care to women in medically underserved communities across the U.S.

- CAP member pathologists' partner with gynecologists, radiologists and other medical professionals to lead See, Test & Treat programs in hospitals, clinics and other facilities

- Women learn the importance of preventive care through annual exams, a Pap test, Mammogram and a healthy lifestyle

See, Test & Treat Needs Your Financial Support

Visit foundation.cap.org and click on DONATE!

THANK YOU!

- Thank you for attending our webinar, **“Preanalytics and the Biospecimen Quality Imperative” by Carolyn Compton, MD, PhD, FCAP**.

- For comments about this webinar or suggestions for upcoming webinars, please contact phcwebinars@cap.org.

- **NOTE:** There is no CME/CE credit available for today’s free webinar. The PDF of the presentation will be sent out in a week.

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COLLEGE of AMERICAN PATHOLOGISTS