Precision Medicine and the Biospecimen Quality Imperative

Carolyn Compton, MD, PhD
Chair, Scientific Advisory Committee, Indivumed GmbH
Professor of Life Sciences, ASU
Professor of Laboratory Medicine and Pathology, Mayo Clinic
Adjunct Professor of Pathology, Johns Hopkins
CMO, National Biomarker Development Alliance
CMO, ASU Complex Adaptive Systems Institute

Northwell Health Grand Rounds
December 8, 2016

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

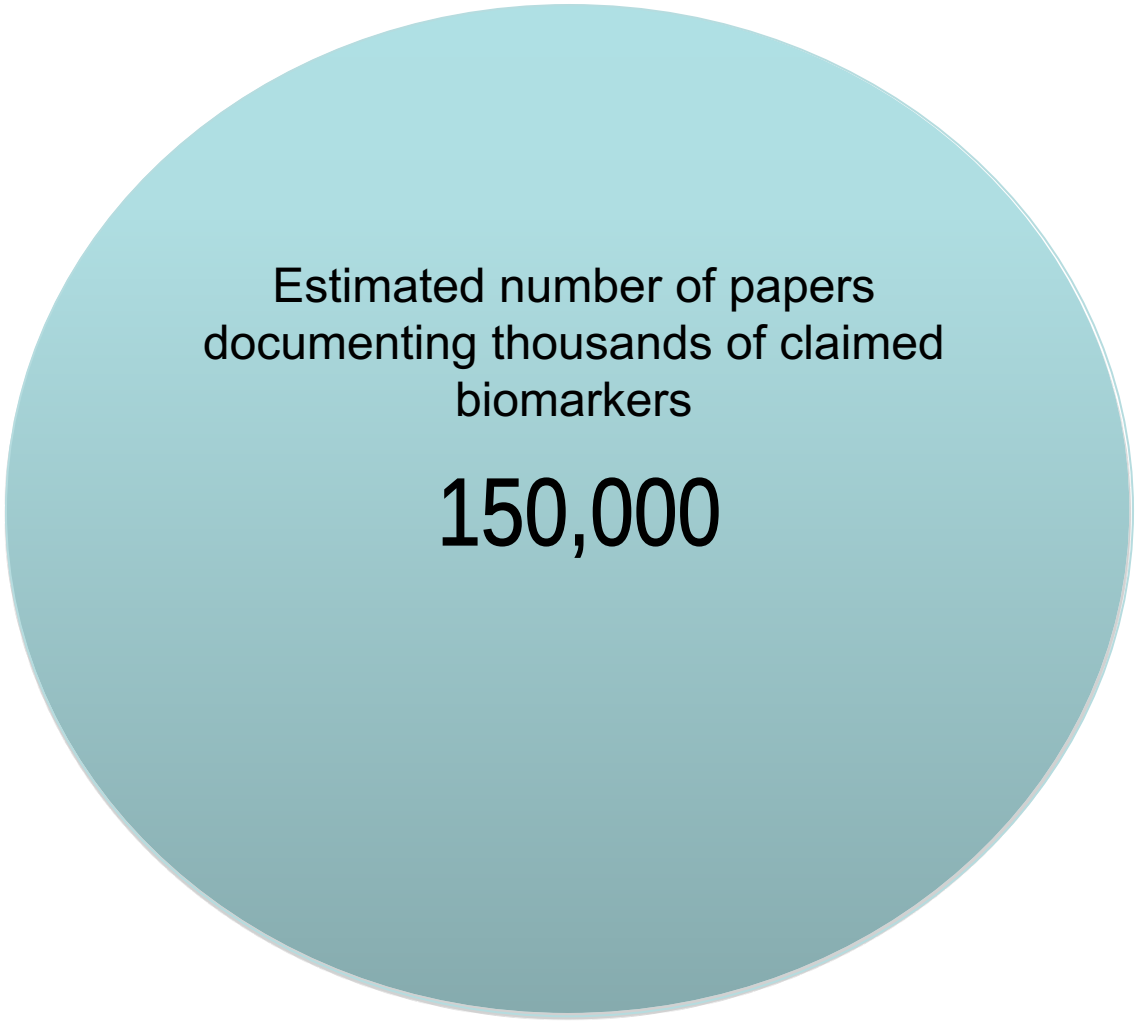
Biomarkers and the Laboratory

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

Usually 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 development – clinical trials: patient selection, efficacy, toxicity, surrogate endpoints

Biomarkers: Many Are Reported, Few Are Qualified



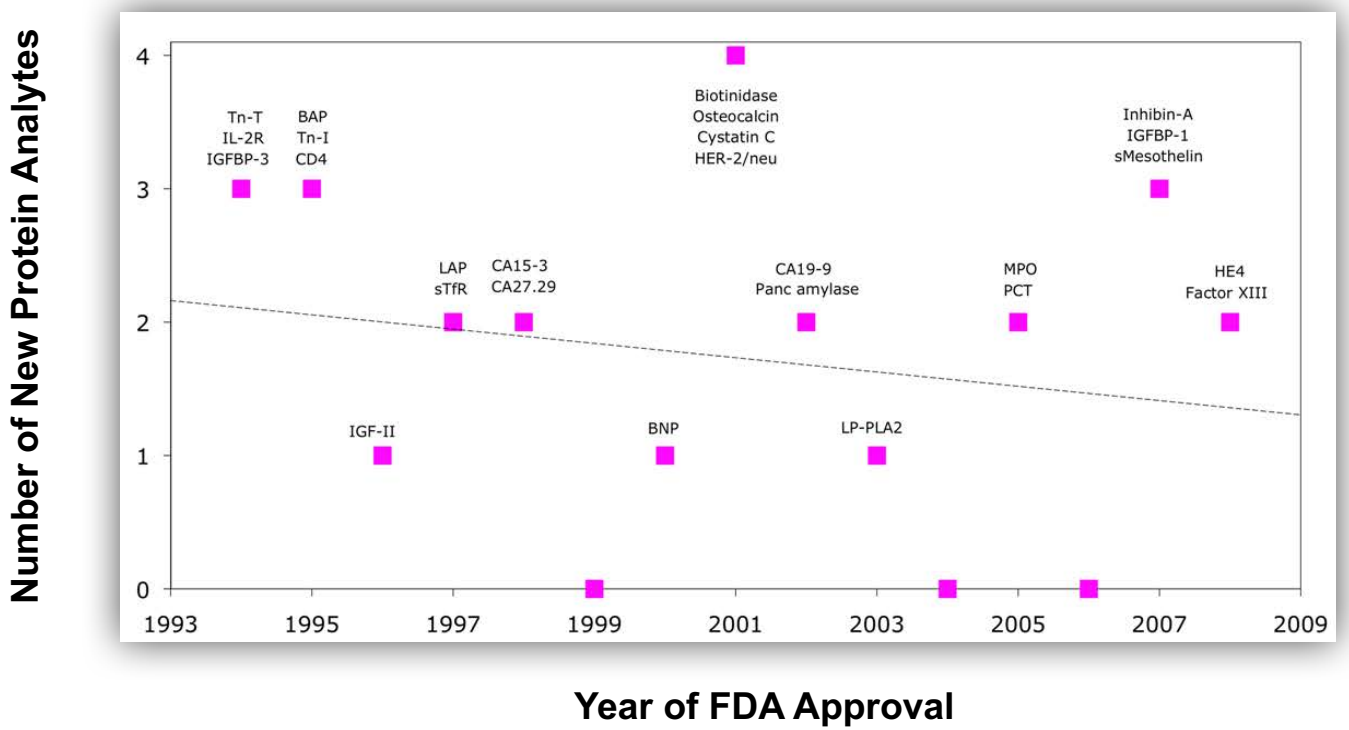
100

Estimated number of biomarkers routinely used in the clinic

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

Sad Status of Protein-Based Biomarkers

- Few biomarker candidates are being approved for clinical use by FDA/EMA
- Approval rate is steadily declining rate



- **Biggest problem is *non-reproducibility* across labs and studies**

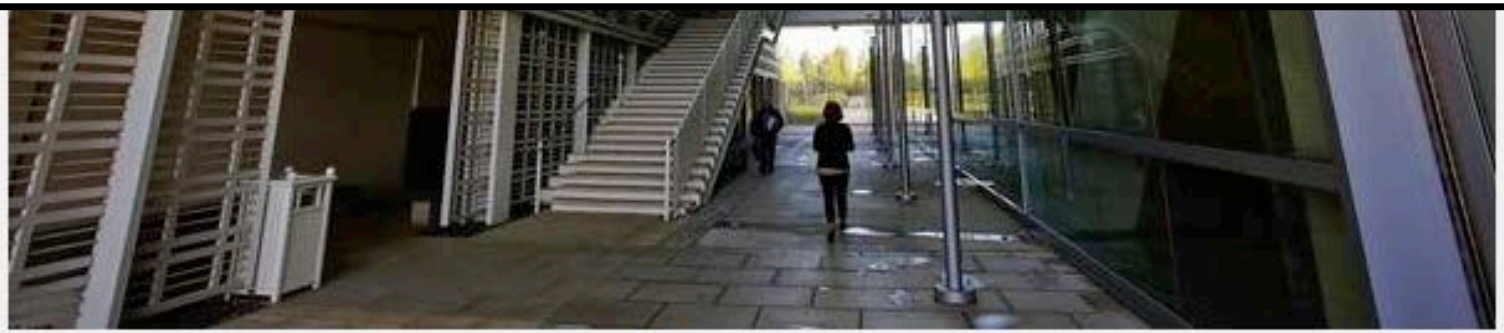
Source: Based on data from FDA and Plasma Proteome Institute

Science has lost its way, at a big cost to humanity

Researchers are rewarded for splashy findings, not for double-checking accuracy. So many scientists looking for cures to diseases have been building on ideas that aren't even true.

Los Angeles Times, October 27, 2013

Amgen attempts to verify results of 53 landmark studies in oncology and hematology;
Only 6 (11%) could be reproduced.



A few years ago, scientists at Amgen set out to double-check the results of 53 landmark papers in cancer research and blood biology. Only six could be proved valid. Above is an Amgen building in Thousand Oaks. (Anne Cusack, Los Angeles Times / April 25, 2013)

How Widespread Are Failures to Reproduce Published Biomedical Science?

- Mass spec diagnostic for ovarian cancer – results due to experimental artifact and bias – control and experimental groups run separately (Lancet, 2002)
- Five of 7 largest molecular epidemiology cancer studies did not classify patients better than chance (JNCI, 96:2004)
- Microarray drug sensitivity signatures – from cell lines – to predict patient response (named one of top100 breakthroughs in 2006) could not be reproduced in large clinical trial in 2009 (Nature Medicine, 2006)
- Of 18 published microarray studies, only 2 were reproducible (Science, 2011)
- Bayer scientists can reproduce only 20-25% of 67 key published experiments and halts 2/3 of its target validation projects as a result (*Nature Reviews Drug Discovery* 10, 712 doi:10.1038/nrd3439-c1, 2011)
- Amgen's team of 100 scientists could reproduce only 11% of 53 seminal studies published on reported drug targets or toxicity (*Nature* 483, 531-533 doi:10.1038/483531a, 2012)

Reproducibility Rate of 10-30% in Academic Biomedical Science

- For biomedical businesses relying on academic discovery to drive product development (like pharma), flipping a coin would be superior to reading *Science* or *Nature* in making business decisions.
- US government spends nearly $31 billion in science funding through the NIH every year, mainly for research grants to academic scientists
  - 10% reproducibility rate ➔ 90% of this money ($28 billion) is wasted
- Wasted money, wasted time, lost opportunities
- Pollution of the biomedical literature by bad studies and bad data:
  - What do we really know? What can we really trust?
- Why should patients and the public believe in what we do?

Irreproducibility in Biomedical Research: A Crisis in Confidence (Public View)

The Economist | World politics | Business & finance | Economics

Unreliable research

Trouble at the lab

Scientists like to think of science as self-correcting. To an ala

Oct 19th 2013 | From the print edition

the Atlantic November 2010

Lies, Damned Lies, and Medical Science

MUCH OF WHAT MEDICAL RESEARCHERS CONCLUDE IN THEIR STUDIES IS MISLEADING, EXAGGERATED, OR FLAT-OUT WRONG. SO WHY ARE DOCTORS—TO A STRIKING EXTENT—STILL DRAWING UPON MISINFORMATION IN THEIR EVERYDAY PRACTICE? DR. JOHN IOANNIDIS HAS SPENT HIS CAREER CHALLENGING HIS PEERS BY EXPOSING THEIR BAD SCIENCE.

By David H. Freedman

PLOS | MEDICINE

Why Most Published Research Findings Are False

John P. A. Ioannidis

Published: August 30, 2005 • DOI: 10.1371/journal.pmed.0020124

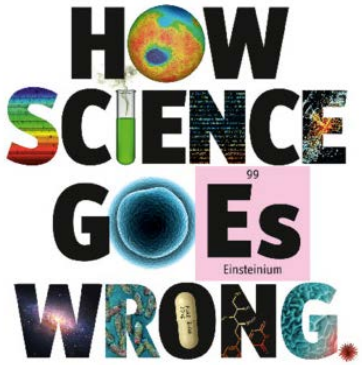
Abstract

Summary

There is increasing concern that most current published research findings are false. The probabili
the number of other studies on the same question, and, importantly, the ratio of true to no relation
framework, a research finding is less likely to be true when the studies conducted in a field are sm
and lesser preselection of tested relationships; where there is greater flexibility in designs, definiti
and other interest and prejudice; and when more teams are involved in a scientific field in chase o
designs and settings, it is more likely for a research claim to be false than true. Moreover, for man
simply accurate measures of the prevailing bias. In this essay, I discuss the implications of these

The Economist

Washington's lawyer surplus
How to do a nuclear deal with Iran
Investment tips from Nobel economists
Junk bonds are back
The meaning of Sachin Tendulkar



THE NEW YORKER

ANNALS OF SCIENCE

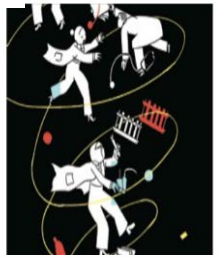
THE TRUTH WEARS OFF

Is there something wrong with the scientific method?

BY JONAH LEHRER

DECEMBER 13, 2010

On September 18, 2007, a few dozen neuroscientists, psychiatrists, and drug-company executives gathered in a hotel conference room in Brussels to hear some startling news. It had to do with a class of drugs known as atypical or second-generation antipsychotics, which came on the market in the early nineties. The drugs, sold under brand names such as Abilify, Seroquel, and Zyprexa, had



December 2011

THE WALL STREET JOURNAL.

WSJ.com

HEALTH INDUSTRY | DECEMBER 2, 2011

Scientists' Elusive Goal: Reproducing Study Results

By GAUTAM NAIK

Two years ago, a group of Boston researchers published a study describing how they had destroy
targeting a protein called STK33. Scientists at biotechnology firm Amgen Inc. quickly pounced o
dozen researchers to try to repeat the experiment with a goal of turning the findings into a drug.

"This is one of medicine's dirty secrets: Most results, including those that appear in top-flight peer-reviewed journals, can't be reproduced"

Irreproducibility in Biomedical Research: A Cultural Norm (Researcher View)

In science, irreproducible research is a quiet crisis

- Few scientists attempt to repeat their own studies
- Publications often based on the one time out of multiple attempts that it actually worked
- External validation (by another lab) is extremely rare
- **Few, if any analyses, focus on the quality and consistency of the biological materials that are the test subjects**

PATRIC SANDRI FOR THE B

By Carolyn Johnson | GLOBE STAFF MARCH 19, 2015

Quality Data Begins with Quality Analytes

Purgamentum init,
exit purgamentum.

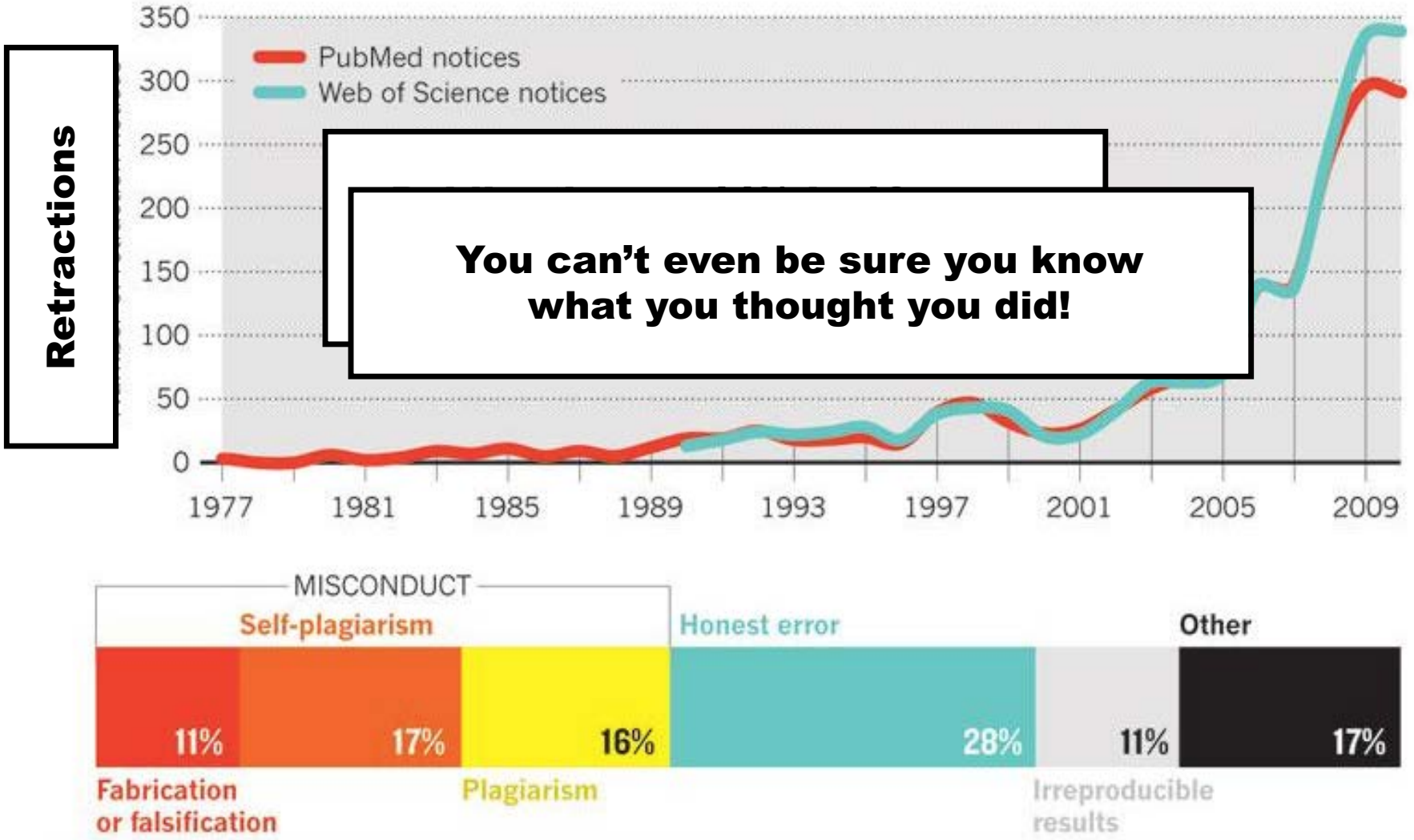
Garbage in…

…Garbage out

Diamonds in……

Modified from Jerry Thomas

Here Today, Gone Tomorrow



White House Takes Notice of Irreproducibility in Science and Seeks Public Input

August 21, 2014

- Federal Register:
- The Office of Science and Technology Policy and the National Economic Council request public comments to provide input into an upcoming update of the *Strategy for American Innovation…….*
- “Given recent evidence of the irreproducibility of a surprising number of published scientific findings, how can the Federal Government leverage its role as a significant funder of scientific research to most effectively address the problem?”

Taking Action

- **Public sector: NIH Rigor and Reproducibility Workshop, 2014**
  - Joint meeting with Science and Nature publishing groups
  - Refers to rigor in use/description of biological reagents (antibodies), cell lines and animals, but **omits reference to human biological materials**
- **Private Sector: The Reproducibility Project**
  - Joint venture between Science Exchange and Center for Open Science
  - Independently replicating a subset of research results from 50 high-impact cancer biology studies published from 2010-2012 using the Science Exchange network of expert scientific labs also **omits reference to human biological materials**

Latest Update

- Yesterday, Congress passed the 21st Century Cures Act
- Increases funding for biomedical research and innovation:
  - $4.8 billion for NIH
  - $500 million for FDA
- Increases funding for FDA
- One provision states that the NIH must convene a working group to develop recommendations for increasing the “rigor and reproducibility” of NIH-funded scientific research and develop or update policies within 18 months.

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

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

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

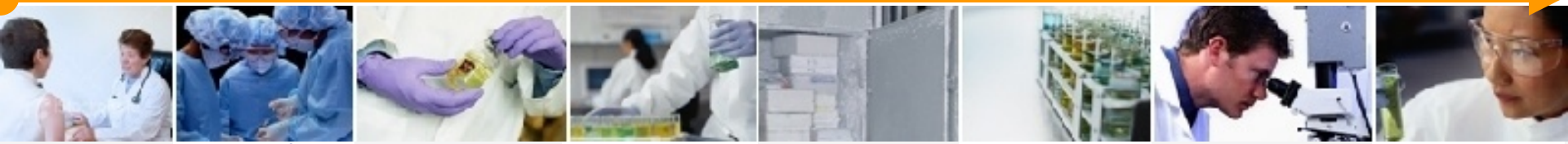
Time 0

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

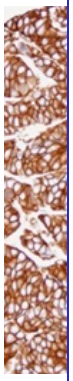
Pre-acquisition

Post-acquisition

Cold Ischemia and Molecular Assay Results

HER2 IHC and FISH in Breast Cancer:
Loss

pMAPK IHC of Colon Cancer :
to



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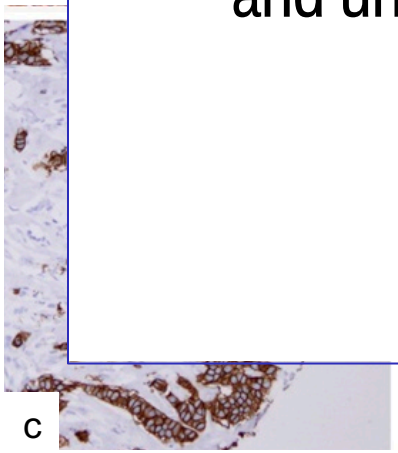

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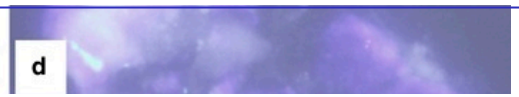

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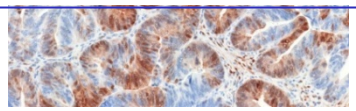


Without knowledge about tissue processing methods and

assurance of rapid tissue fixation,

protein expression data are unreliable,

and understanding of pathway activity is impossible.

- Hartmut Juhl, CEO

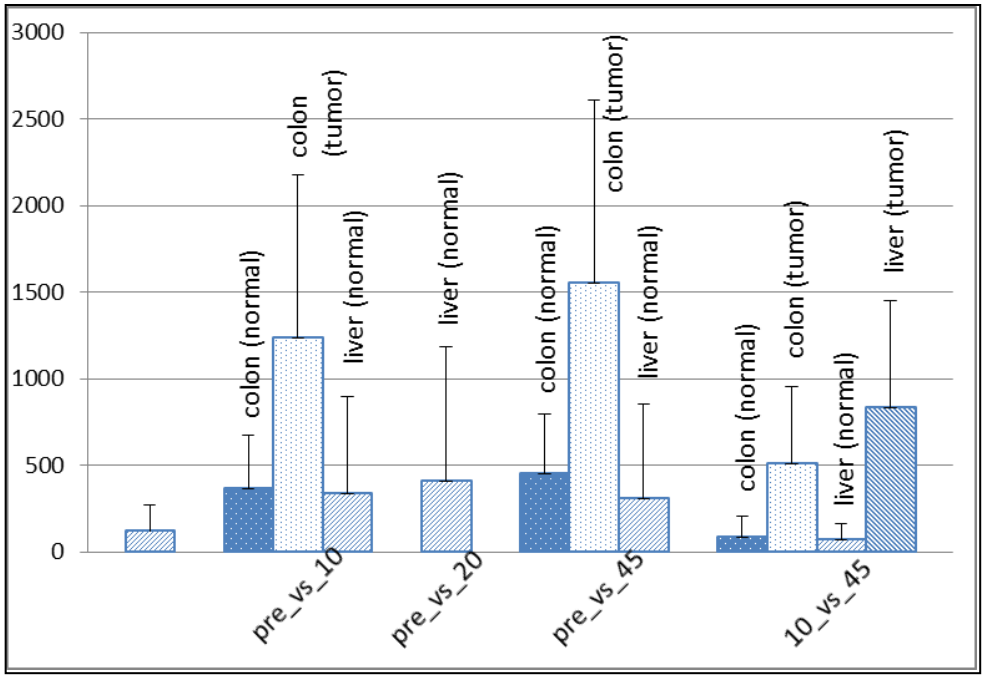
Indivumed

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

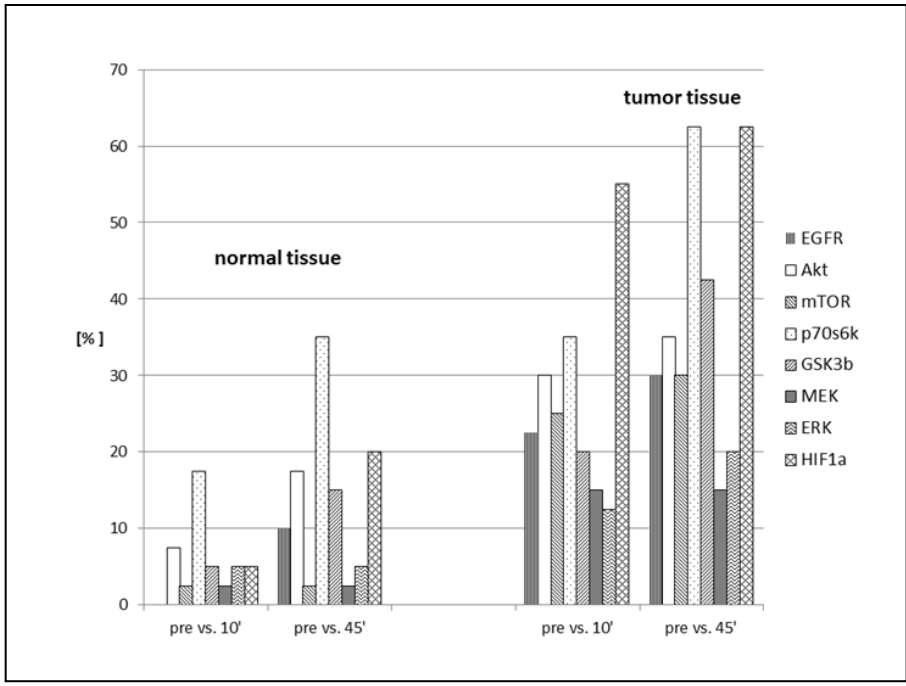
Hartmut Juhl, Indivumed GmbH, BRN

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

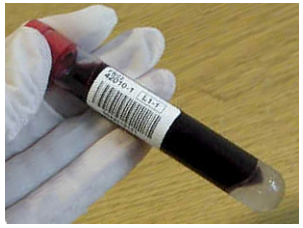
Gene Expression
Pre vs. Post Surgery



Protein Expression
Pre vs. Post Surgery



Blood Collection and Plasma Processing: Biomarkers and Circulating Tumor Cells



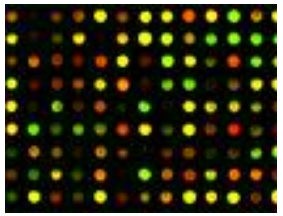
Collection Tubes and Order of draw

Processing Procedure, Temperature and Time

Distribution & Storage

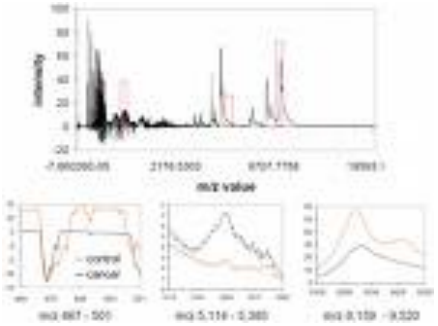
Blood Draw Procedure

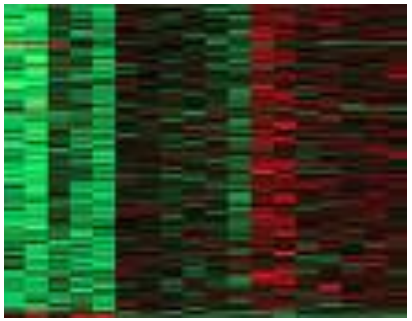
Patient Consent and Preparation



Molecular Analysis

Biospecimen Quality Impacts Both Clinical And Research Outcomes





Effects on Clinical Outcomes

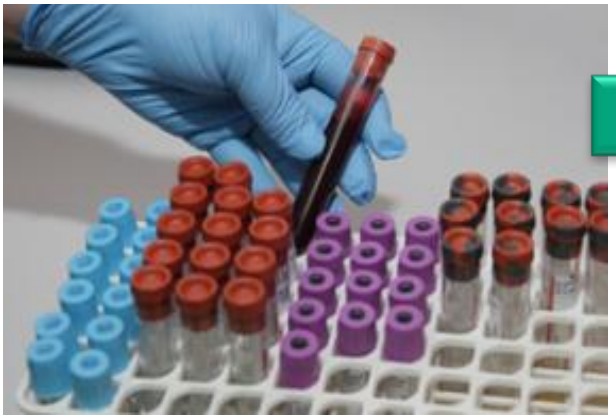
- Potential for incorrect diagnosis
- Potential for incorrect treatment
  - Therapy linked to diagnostic test on a biospecimen

Effects on Research Outcomes

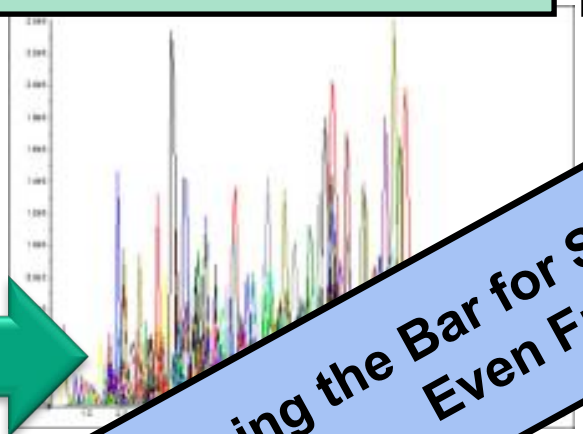
- Irreproducible results
  - Variation in mutation data
  - Variation in gene expression data
  - 11-25% reproducibility of published biomedical data
- Misinterpretation of artifacts as biomarkers

Evolution of Biomarker Testing

Unianalyte Tests



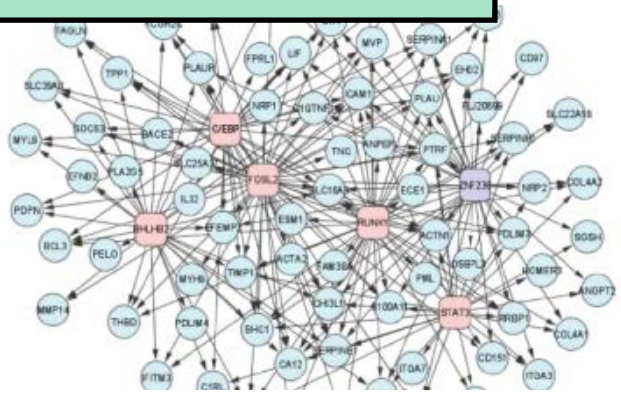
Multianalyte Tests



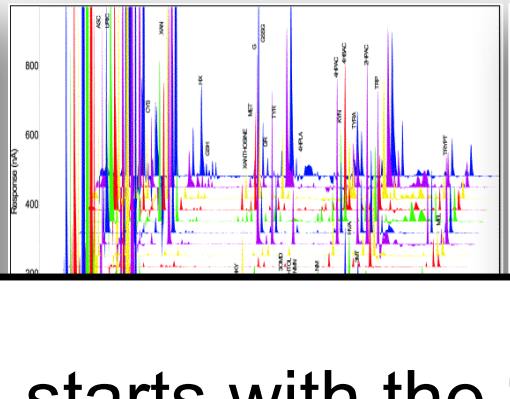
Omics Analyses

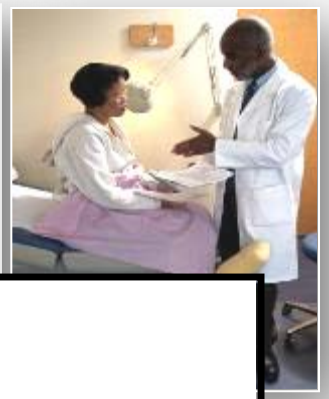
Raising the Bar for Specimen Quality Even Further!

Networks and Systems

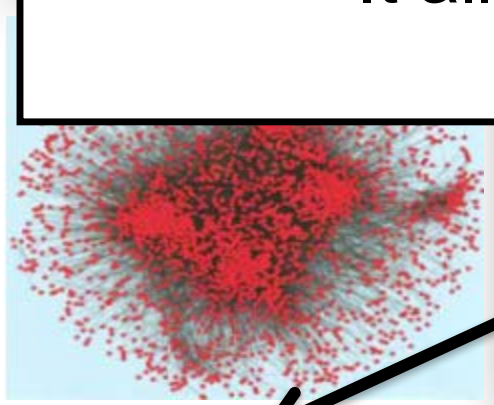
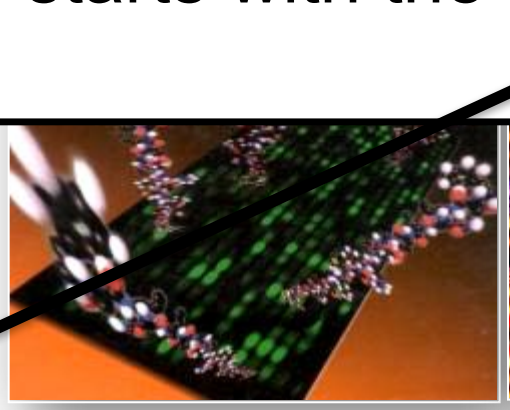
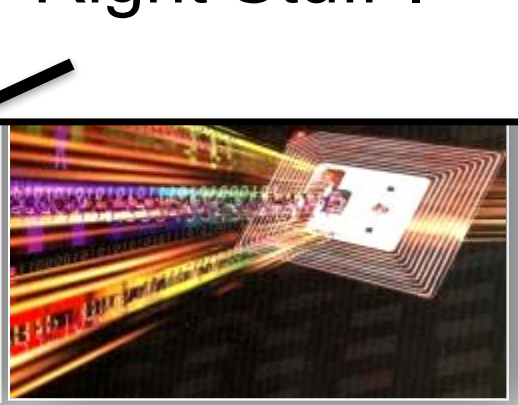


And It's Getting Far More Challenging



It all starts with the "Right Stuff".


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!
- 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

Molecular Analysis for Therapy Choice: The NCI MATCH Trial To Link Targeted Cancer Drugs to Gene Abnormalities

IN THE LAB

Shoddy biopsies deny cancer patients a shot at personalized treatment

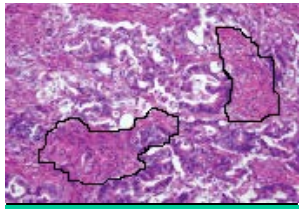
DAN KITWOOD/GETTY IMAGES/CANCER RESEARCH UK

Shoddy tumor biopsies are preventing cancer patients from receiving personalized therapies.

By ELIE DOLGIN @eliedolgin

JANUARY 22, 2016

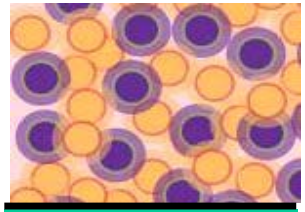
The Right Answers Depend on the Right Stuff



% Tumor content

Tumor cells are typically mixed with normal tissue.

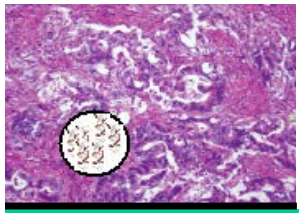
Tumor content may be enriched by macro-dissection



% Mutant copies

Tumors have background of wild-type DNA.

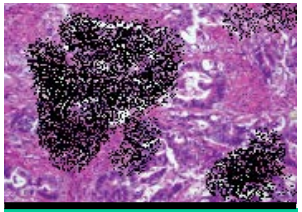
Challenge to detect low % mutant alleles



% Amplifiable DNA

Tissue fixation damages DNA.

Necrotic cells may not have amplifiable DNA



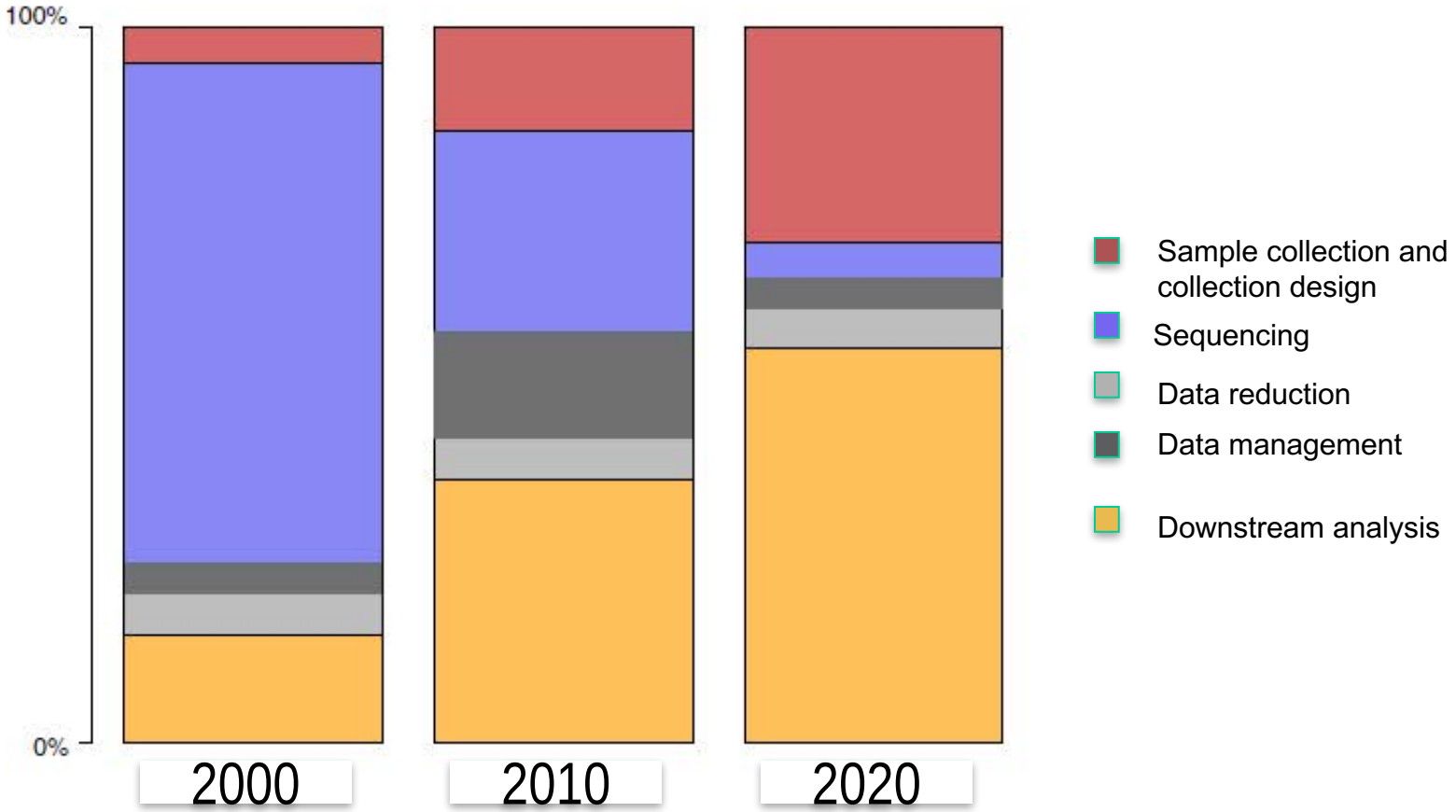
% Fixation inhibitors

Natural and introduced inhibitors may interfere with amplification

FFPET = formalin-fixed, paraffin-embedded tissue

Estimating the Changing Aspects of NGS

Are pathologists prepared for what's coming?



From Ken Bloom, MD, GE Healthcare, June 2014

Biomarker Development Is a Team Sport

NBDA: Understanding the Issues in Biomarker Development and Building Solutions

The Process of Biomarker Development Is Siloed and Fragmented

Early Discovery
(Biology Verified Patient Samples)

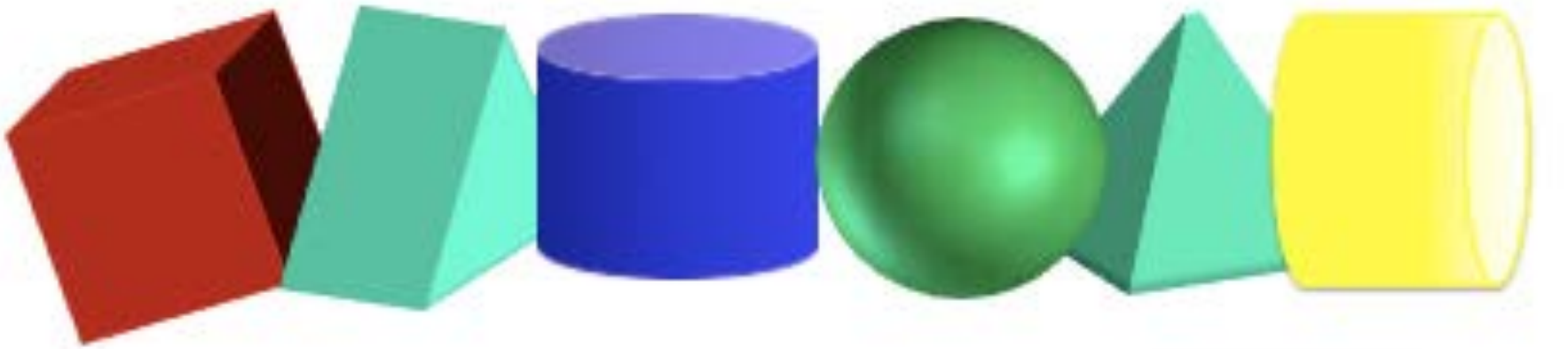
Translatable Discovery
(Clinical Measure Established)

Assay Development
(Analyte - Reagents- Technology – Robust)

Assay Performance
(Analytical Validation)

Biomarker Qualification
("Fit for Clinical Purpose)

Biomarker Validation
(Clinical Validation)



Academia

Industry

Physicians

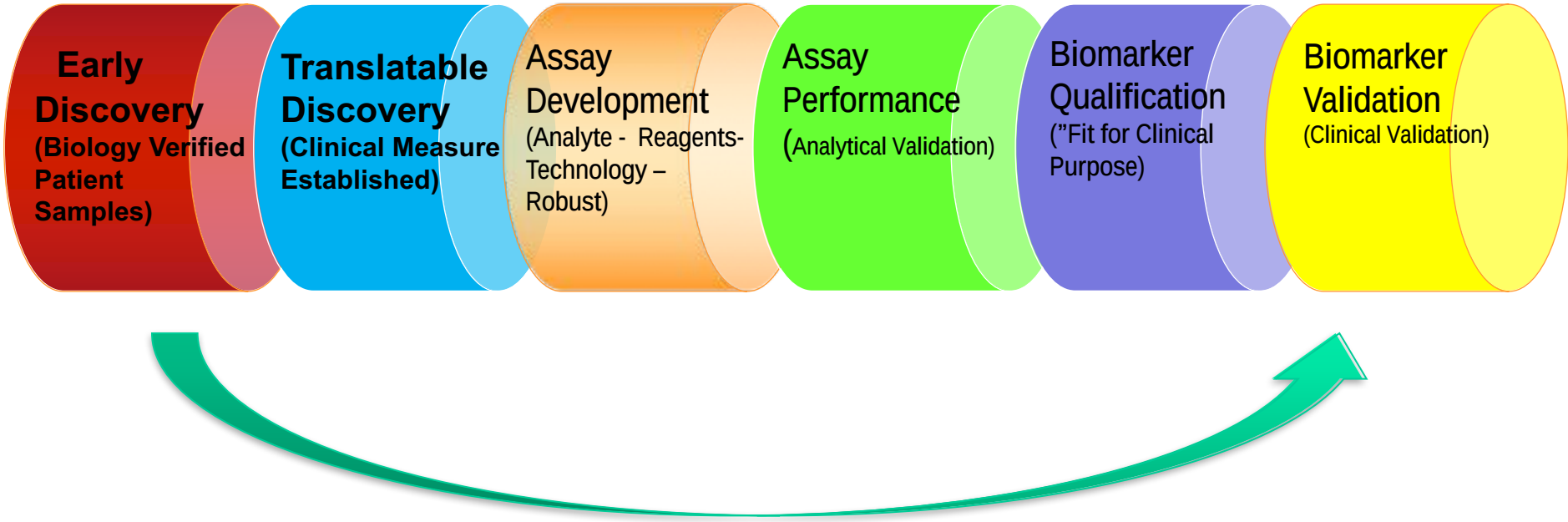
Patients

Regulators

Funding Agencies

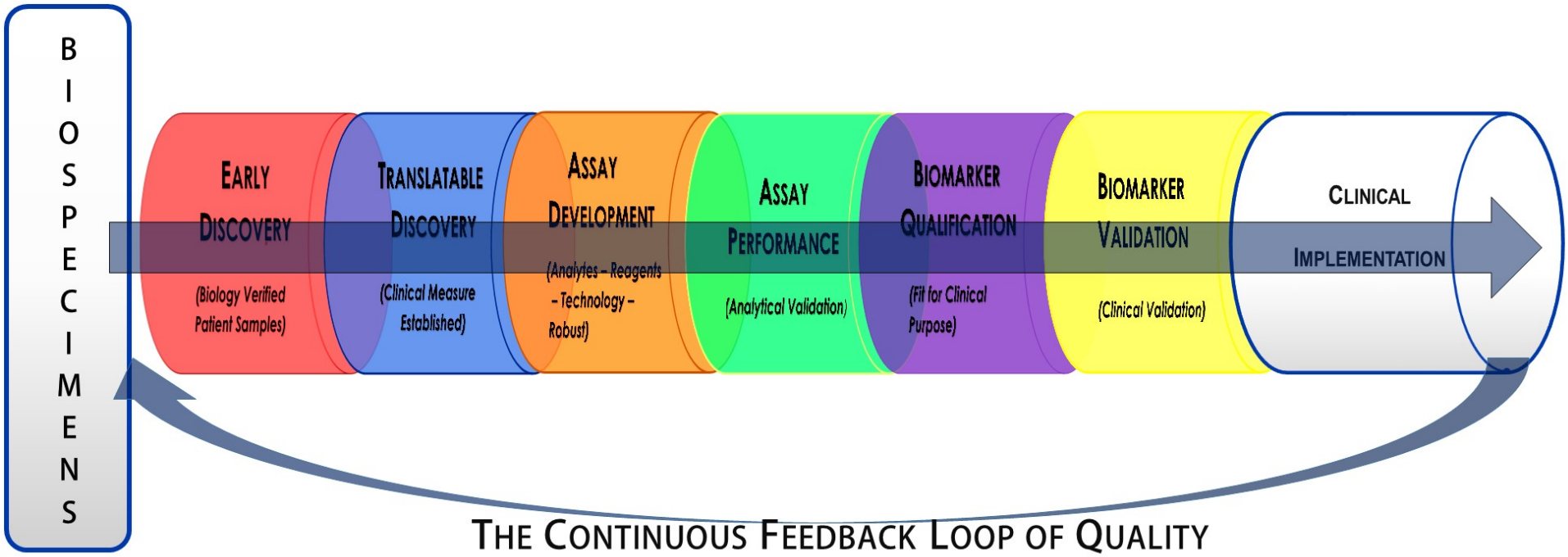
Professional Bodies

Realizing an End-To-End, Standards-Based Approach to Biomarker Development

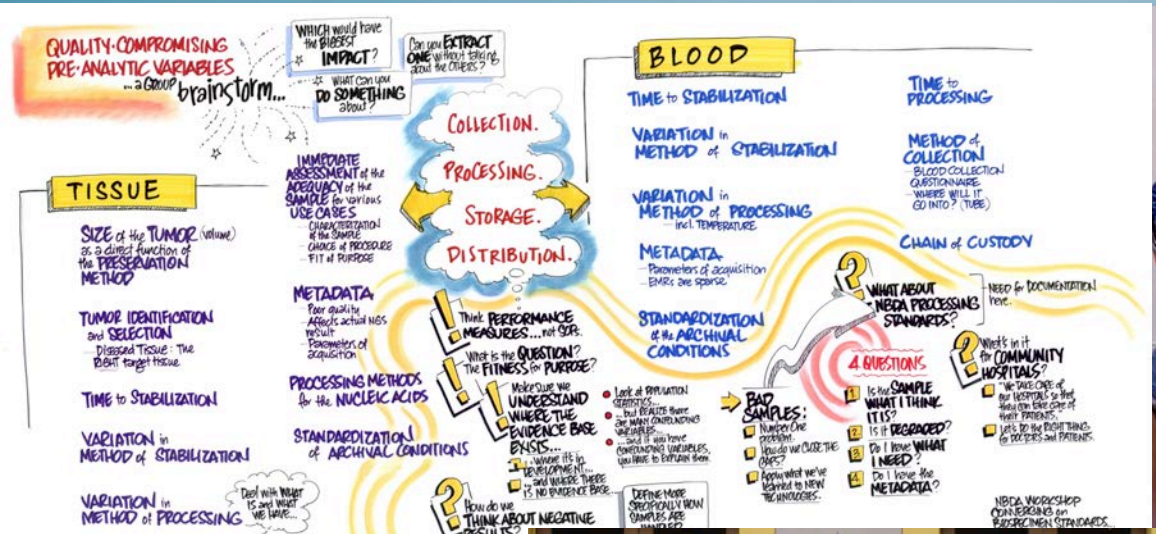


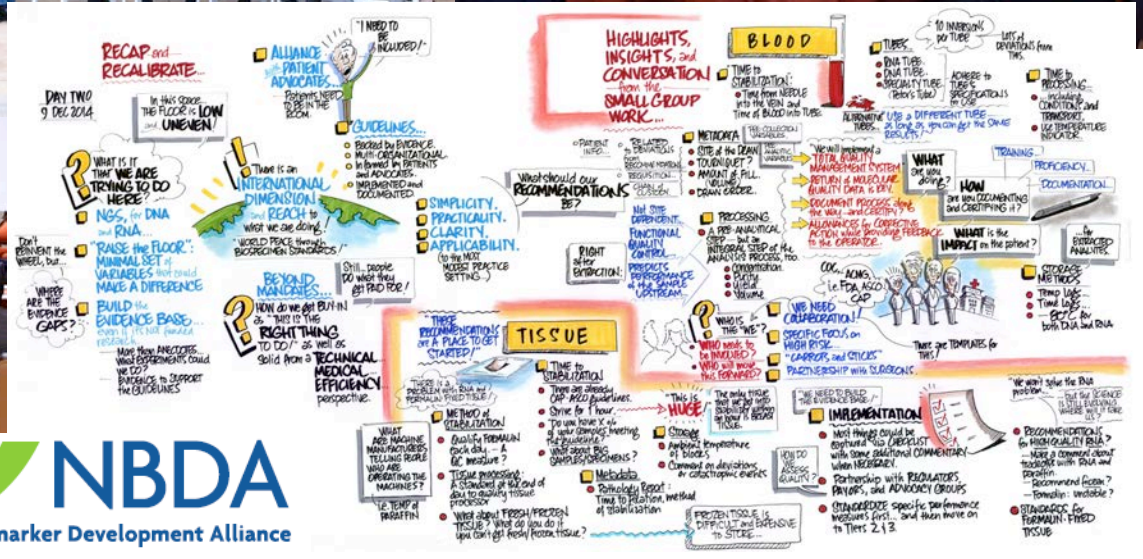
Standards are needed at every step and across the continuum

Biospecimens Flank End-To- End Biomarker Development



NBDA: Understanding The Issues - Building Towards Solutions





NBDA
National Biomarker Development Alliance

55 Attendees – Representing All Stakeholder Groups and Points of View

- Academic genomics experts (scientists: basic and translational)
- Academic proteomics experts (scientists: basic and translational)
- Expert molecular pathologists
- CAP leadership:
  - President
  - President Elect
  - Immediate Past President
- Surgeons
- Patient advocacy group leaders: JDRF
- Funders: NCI
- Regulators: FDA
- Leadership of professional societies: ASCO, AACR
- Payers: CMS, Palmetto, Aetna, BC/BS
- Industry (Pharma, Platform manufacturers, Tissue providers): Illumina, Genetech, Caprion, Indivumed, Becton-Dickenson, Novartis, Abbott)

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*)

NBDA Genomics Convergence Conference: Defining a Benchmark for Patient Biospecimens

Pareto Principle (20/80 rule)

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

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
   - Draw parameters (needle, vein vs. line)
   - Volume of tube fill
3. Method of stabilization
   - Tube type (stabilizer preset or not)
   - Tube inversions
4. Method of processing
   - Centrifugation speed/time
   - Temperature
5. Storage conditions
   - Freeze/thaw cycles
6. (Metadata to be collected]

Action

- Pre-analytics for Precision Medicine Project Team: College of American Pathologists
- Verification of the Top 5 lists for Tissue and Blood Specimens from NBDA Convergence: literature review, CLIA, ISBER, NCI
- Develop a Top 5 for cytology specimens
- Establish performance metrics around the Top 5's
  - DATA-DRIVEN
  - PRACTICAL
- Educate pathology workforce (pathologists, pathology assistants, medical laboratory technicians, phlebotomists)
- Implement and enforce performance metrics through the CAP Laboratory Accreditation Program checklists
- Seek new reimbursements codes, if needed
- Seek reinforcement through FDA guidance, research funder requirements

CAP Validated Practicable Benchmarks: Tissue

1. Time to stabilization: **60 minutes. or less**
2. Method of processing
   - **Section thickness:** ≤5 mm
   - **Mass/volume formalin 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

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.

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

Specimen Quality Is A Front-loaded Issue

"If you don't have the time to do it right,
when will you have the time to do it over?"

- John Wooden, Coach UCLA

Our Challenge

Garbage in…

…Garbage out

Precision Medicine and the Biospecimen Quality Imperative

Carolyn Compton, MD, PhD
Chair, Scientific Advisory Committee, Indivumed GmbH
Professor of Life Sciences, ASU
Professor of Laboratory Medicine and Pathology, Mayo Clinic
Adjunct Professor of Pathology, Johns Hopkins
CMO, National Biomarker Development Alliance
CMO, ASU Complex Adaptive Systems Institute

Northwell Health Grand Rounds
December 8, 2016