

EPI 246:

Applied Biomarkers in Cancer

Epidemiology

October 24, 2017

Heather Eliassen, ScD

Overview

- Objectives and overview of course
- Instructor & student introductions
- Course requirements
- Whirlwind review of Epi 240: Biomarkers in Epidemiology Research

Objectives of course

- Provide an organizational framework to evaluate different types of biomarkers in the context of cancer epidemiology and prevention
 - Categorize biomarkers along the continuum of exposure to cancer
 - Assess biomarker studies within these categories
- Provide detailed examples of a variety of different biomarkers and their contribution to the field of cancer epidemiology

Objectives of course

- Students will develop skills to critically evaluate biomarker subtype, study design, strengths and weaknesses, and contribution to field:
 - Individual write-up of paper for discussion
 - Peer review of article
 - Discussion

In addition...

- To reduce overlap, minimized use of
 - Nutritional examples
 - HPFS and NHS cohort examples
- Cover a wide-range of cancers
- Discuss different types of study designs

What is a biomarker?

Types of biomarkers

- Risk biomarkers
- Surrogate endpoint
- Efficacy or outcome biomarkers
- Mechanism biomarkers
- Pharmacodynamic biomarkers
- Target biomarkers
- Toxicity biomarkers
- Translational biomarkers

Nature Biotechnology, March 2005

Biomarkers in cancer research

Areas of application:

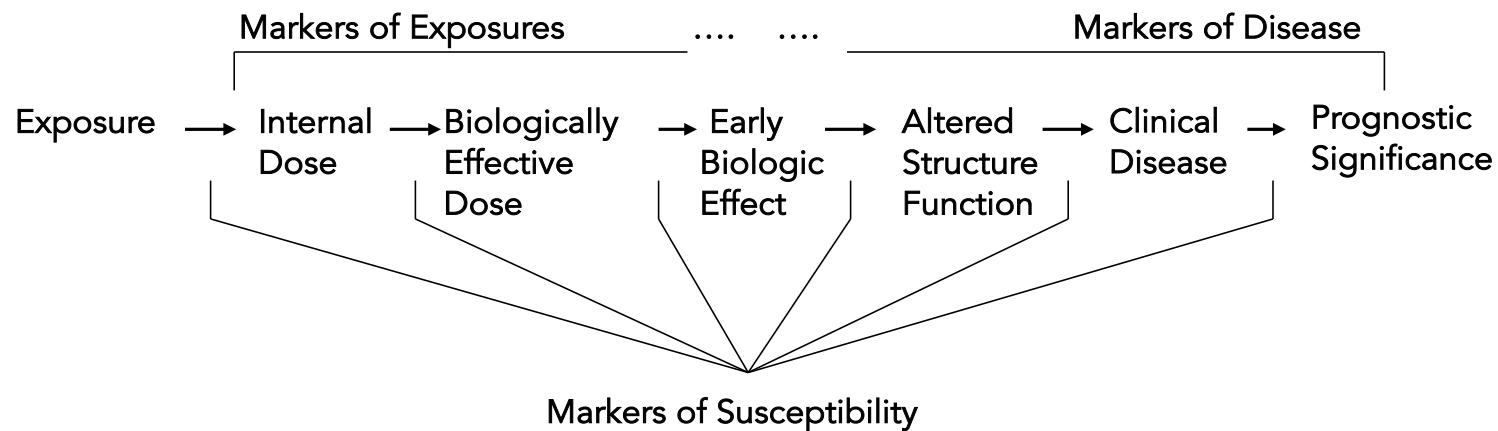
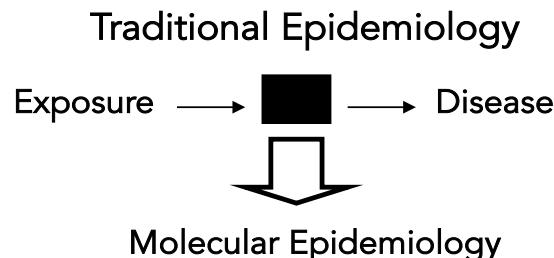
- Research on etiology of cancer
- Disease mechanisms and pathogenesis
- Cancer prevention: screening, intervention trials
- Clinical trials (treatment)
- Validation studies (e.g., correlation between external and internal dose)
- Susceptibility and gene-environment interaction

Biomarkers in cancer epidemiology

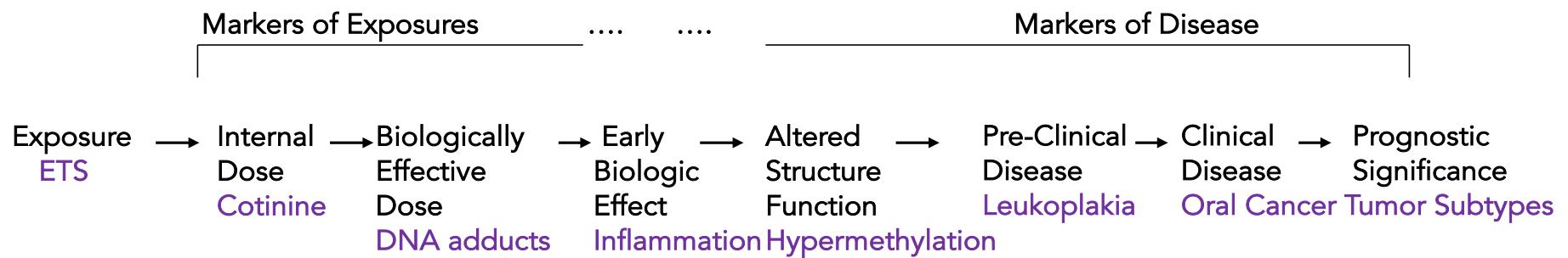
Purpose is to:

- Provide mechanistic understanding
- Increase precision/reproducibility
 - Should also decrease number of studies needed
- Detect cancers at early stages
 - For treatment and better survival
 - Screening
 - To provide endpoints for Phase II trials and increase efficiency of design

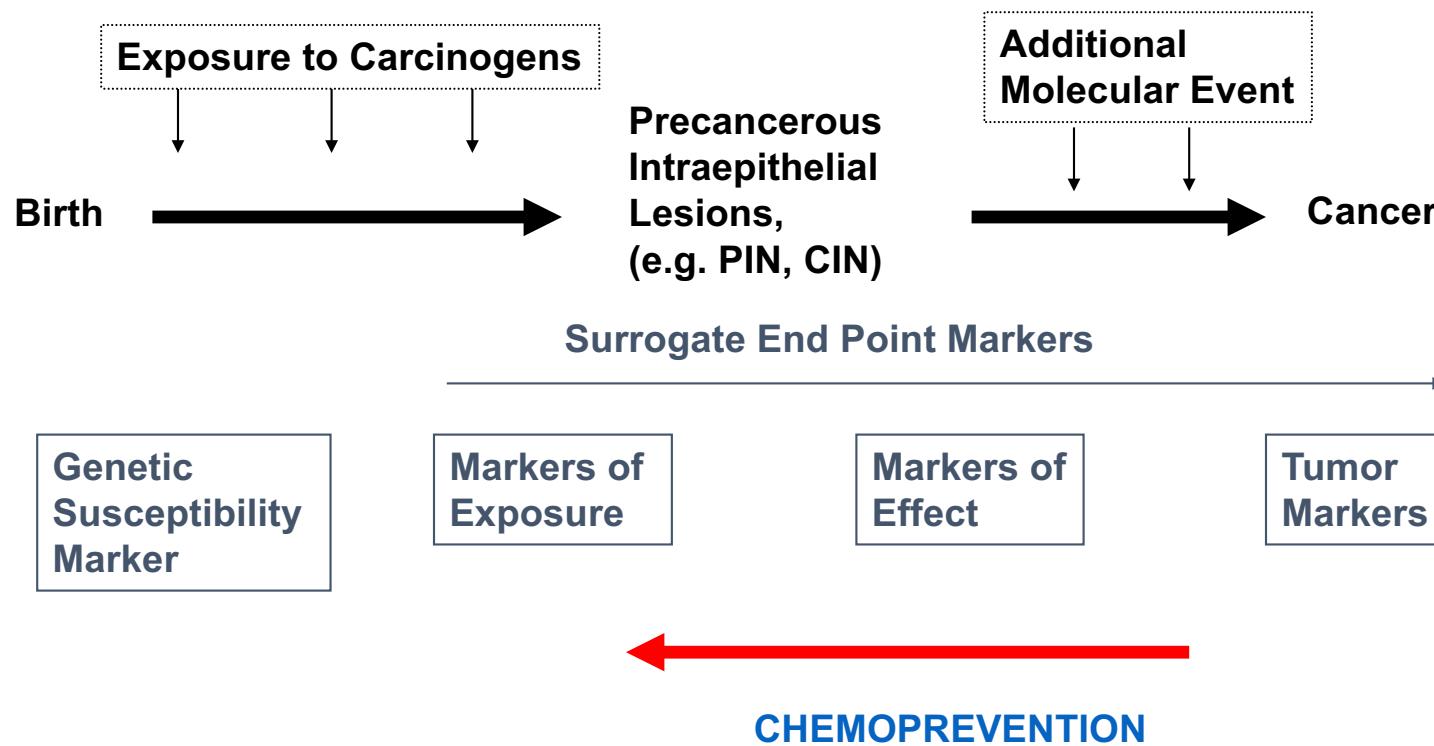
Biomarker categories along the continuum from exposure to cancer



Biomarker categories along the continuum from exposure to cancer: examples



Biomarkers on the cancer continuum



Surrogate endpoints

- Biomarkers used as a surrogate for incident cancer in observational and clinical studies
- A surrogate endpoint biomarker is generally closer to the cancer endpoint on the continuum scheme (e.g., pre-cancer)
- A surrogate endpoint biomarker can also be used for early detection

Assessing biomarker studies

- Aim(s) of study
- Study design
- Biomarker validity
- Assay validity (QC inclusion)
- Strengths and limitations
- Existing knowledge in field

Instructor & Student Introductions

Heather Eliassen, ScD

HARVARD SCHOOL OF PUBLIC HEALTH PAGE 1 Nurses' Health Study II

1. PLEASE USE PENCIL
CURRENT WEIGHT

2. Do you currently smoke cigarettes?
 No Yes Never smoked 1 to 14 15 to 44 45+ 50+ 55+

3. Have yo
 No Yes I don't know

4. Have yo
 No Yes I don't know

5. Have yo
 No Yes I don't know

6. Are you
 Not at all Somewhat Quite a bit Very much

7. Since June 2013, have you used
 Yes No
 a) How many months
 0-1 month 1-4 months 4-12 months 1-2 years 2-5 years 5-10 years 10-15 years 15-20 years 20+ years
 b) Are you currently taking
 No Yes
 c) Mark the type(s) of combined:
 Progesterone/Estrogen: Oral Premarin or conjugated estrogen Estrogen gels, creams, or sprays Provera/Cyndrome/MPA
 Progestrone: Other progestrone
 Other hormones: Bioidentical estrogen
 Contraceptive: Bioidentical progesterone
 d) If you used oral conjugated estrogen (e.g., Premarin), how many mg/day or less
 0-10 mg/day 11-20 mg/day 21-30 mg/day 31-40 mg/day 41-50 mg/day 51-60 mg/day 61-70 mg/day 71-80 mg/day 81-90 mg/day 91-100 mg/day 101-110 mg/day 111-120 mg/day 121-130 mg/day 131-140 mg/day 141-150 mg/day 151-160 mg/day 161-170 mg/day 171-180 mg/day 181-190 mg/day 191-200 mg/day 201-210 mg/day 211-220 mg/day 221-230 mg/day 231-240 mg/day 241-250 mg/day 251-260 mg/day 261-270 mg/day 271-280 mg/day 281-290 mg/day 291-300 mg/day 301-310 mg/day 311-320 mg/day 321-330 mg/day 331-340 mg/day 341-350 mg/day 351-360 mg/day 361-370 mg/day 371-380 mg/day 381-390 mg/day 391-400 mg/day 401-410 mg/day 411-420 mg/day 421-430 mg/day 431-440 mg/day 441-450 mg/day 451-460 mg/day 461-470 mg/day 471-480 mg/day 481-490 mg/day 491-500 mg/day 501-510 mg/day 511-520 mg/day 521-530 mg/day 531-540 mg/day 541-550 mg/day 551-560 mg/day 561-570 mg/day 571-580 mg/day 581-590 mg/day 591-600 mg/day 601-610 mg/day 611-620 mg/day 621-630 mg/day 631-640 mg/day 641-650 mg/day 651-660 mg/day 661-670 mg/day 671-680 mg/day 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Eva Schernhammer, MD, DrPH



- Adjunct Associate Professor TH Chan School
- Professor of Epidemiology, Head of Department of Epidemiology, Medical University of Vienna



Guest Lecturers

- Brenda Birnbaum, ScD; Channing/TH Chan
- Rulla Tamimi, ScD; Channing/TH Chan
- Immaculata DeVivo, PhD; Channing/TH Chan
- Roopali Roy, PhD; Boston Children's Hospital
- Curtis Huttenhower, PhD; TH Chan
- Lorelei Mucci, ScD; TH Chan
- John Quackenbush, PhD; Dana Farber/TH Chan

Student Introductions

Course Requirements

EPI 246

Fall 2017

Grading

- Class discussion: 10%
- Written reports of 2 articles: 15% each
- Oral presentation of 1 of the 2 articles: 20%
- Peer review of article: 40%

Written report of 2 articles

- Total 30% of grade
- Max. 3 Pages
 - Brief summary of background (max. 1 p)
 - Methods, Results, Strength, Limitations (max. 1-2 p)

Oral presentation

- 20% of grade
- 5-minute presentation of 1 of the 2 written reports
- Slides to be submitted 1hr before class

Class discussion

- What are the strengths? Weaknesses?
- Big picture: How did this study contribute to understanding of cancer or cancer prevention?
 - Novelty of finding, design, and/or biomarker?
 - Improved biomarker, methodology (including power), and/or assay?
- Discussion questions circulated prior to class
- Every student must be prepared to answer questions and is expected to read articles prior to EVERY class!
- Attendance is mandatory
- Emphasis of class participation is on participation in the discussion and answering questions

Peer-review of article (Midterm)

- Constitutes 30% of grade
- You will be asked to review an article
- Based on your expert opinion, you will decide whether to accept, revise, or reject the manuscript and submit a critical review with a response to Editors and with constructive suggestions for improvements to the Authors
- The article will be an earlier publication and will have to be reviewed in the context of research as it stands today
- Students must work on their own for this exercise!

Course evaluation

- The submission of a course evaluation (online) is a requirement for this course
- We will ask you for an evaluation of our guest speakers at the end of the course

Contact information

- Heather: heather.eliassen@channing.harvard.edu
- Eva: eva.schernhammer@channing.harvard.edu

Let's have fun!

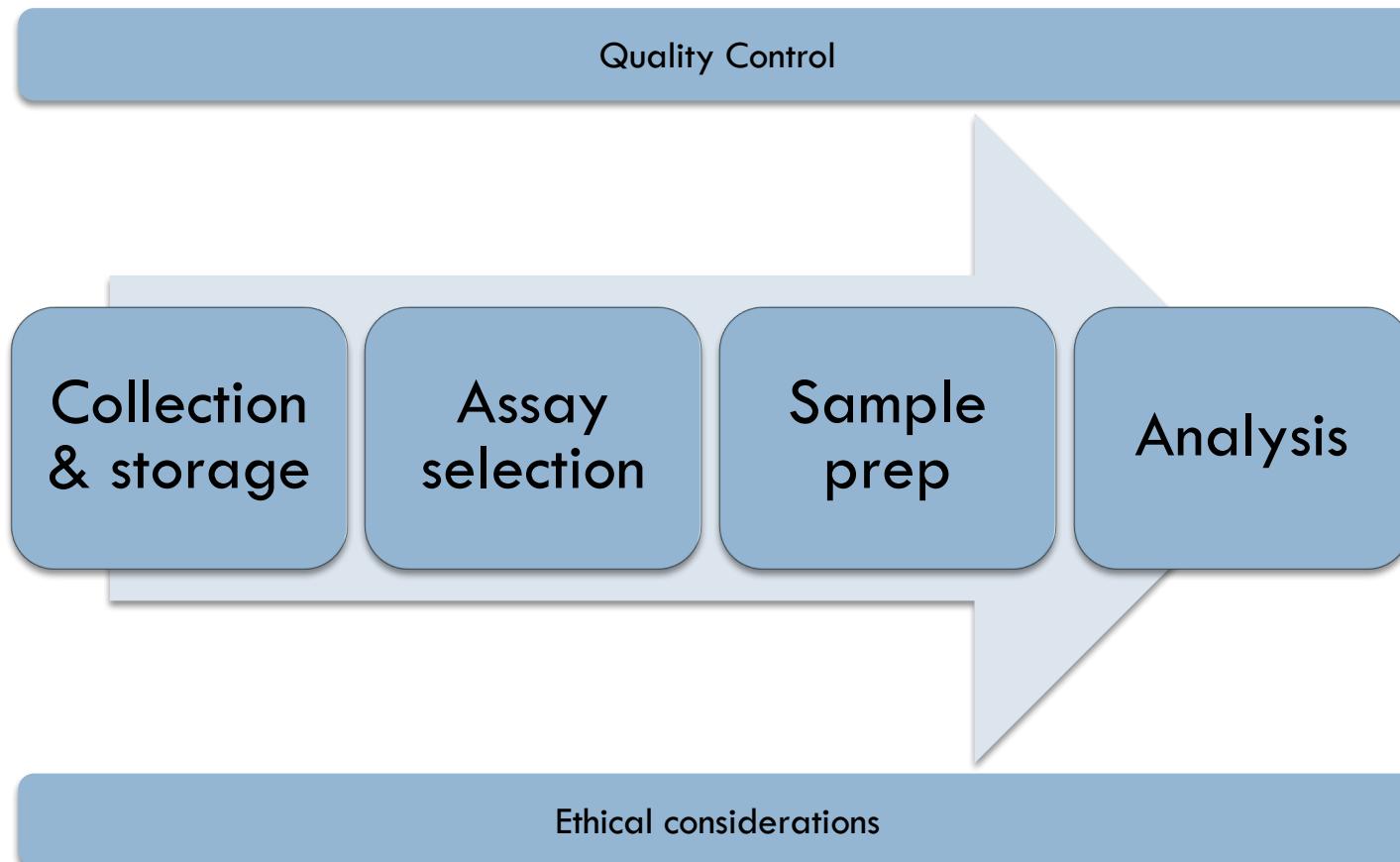
Biomarkers in Epidemiology Research

Brief summary of relevant material covered in EPI 240

Readings for today

- Vineis and Perera, 2007. Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. *CEBP*; 16:1954-1965.
- Tworoger and Hankinson, 2006. Collection, processing, and storage of biological samples in epidemiologic studies: sex hormones, carotenoids, inflammatory markers, and proteomics as examples. *CEBP*; 15(9):1578-81.
- Optional reading: Tworoger and Hankinson, 2006. Use of biomarkers in epidemiologic studies: minimizing the influence of measurement error in the study design and analysis. *Cancer Causes Control*; 17(7):889-99.

Lifecycle of a biomarker study



Overview

- Biomarker and assay validity
- Collection, processing, storage of biospecimens
- Measurement error in assay

Biomarker and Assay Validity

Biomarker validity

- The accuracy with which the biomarker measures the underlying, biologically-relevant phenomenon of interest

Biomarker validity

- Does biomarker actually measure the exposure of interest?

Exposure of interest	Measured biomarker
Hormones in breast or other tissue	Circulating hormones
Gene activity	Genetic polymorphism
DNA repair capacity	Assess DNA damage after exposure to mutagens

Assay validity

- The accuracy with which the assay used measures the biomarker of interest
- Is the assay measuring the biomarker?
- Can be difficult to determine a gold standard
 - Gold standard is necessary to get a direct assessment of validity
 - For some serum or plasma assays mass spectroscopy is considered gold standard
- Data from a reliability study can be used to correct for non-differential measurement error

Assay attributes

- Assay sensitivity
 - The lowest concentration of a biomarker that is distinguishable from background or negative control
 - Limit of detection: The lowest and highest concentrations that the assay can reliably detect
- Assay specificity
 - The capability of an assay to differentiate similar analytes or interference from matrix elements

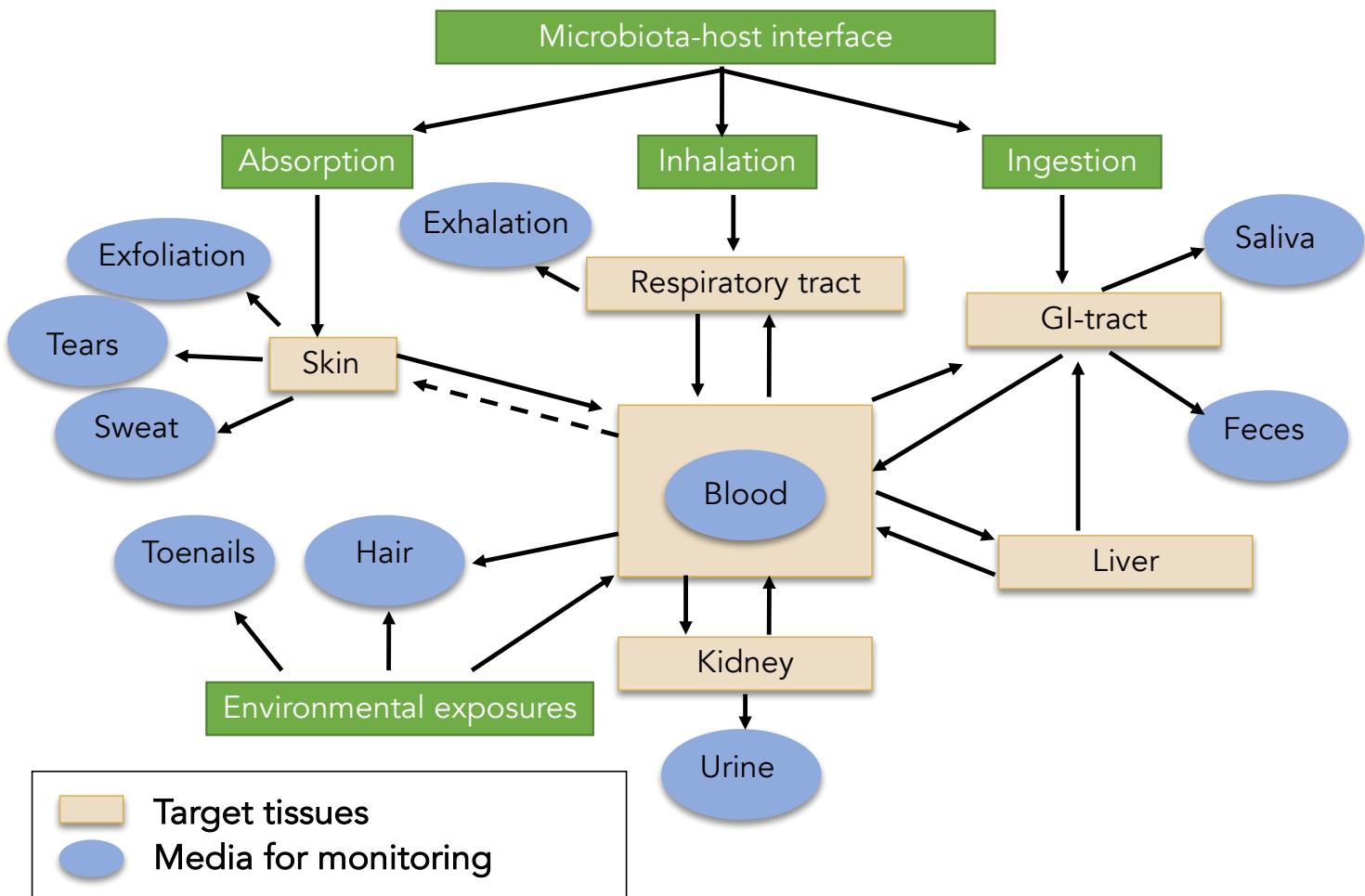
Collection, Processing, Storage of Biospecimens

Who to collect from

- Depends on scientific question
 - Disease?
 - Exposure?
- Must have variation in biomarker
 - Won't work with no between person variation
- Population must be willing to give the samples
 - Pilot!

What to collect

- More than one sample type for flexibility in future research
- Scientific question of interest
 - Time integration
 - Biomarkers of interest
- Feasibility in your population
 - Will healthy people be able to contribute?
 - Cases may have problems giving sample



Adapted from Committee on Biological Markers, National Research Council. Biological Markers in Environmental Health Research. *Environ Health Perspect* 1987;77:3-9.

How to collect

- Most sample types have many ways to collect
- How you collect can impact:
 - Which biomarkers can be assessed
 - How much sample can be obtained
 - Price
 - Long term usability
- Again, consider population of interest: can/will participants comply?

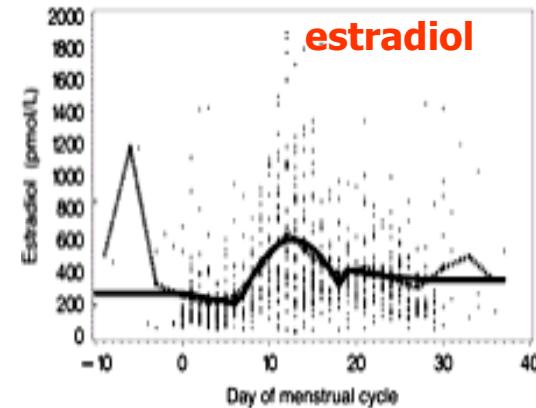
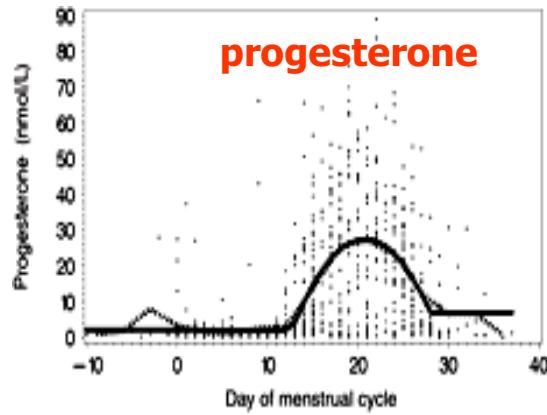
Types of sample collections

- Blood
 - Venipuncture
 - Finger stick
- Urine
 - Cup
 - Filter paper
- Breast nipple aspirate
 - Ductal lavage
 - Needle
- Tumor tissue
 - Fresh frozen
 - Paraffin-embedded
- Saliva / cheek cells
 - Timed/untimed
 - Passive drool
 - Stimulation (e.g., gum)
 - "Swish and Spit"
 - Spit, with RNA later
 - Buccal brush
 - Filter paper
- Stool
 - Fresh frozen
 - FOBT card
 - 95% Ethanol
 - RNA later

When to collect

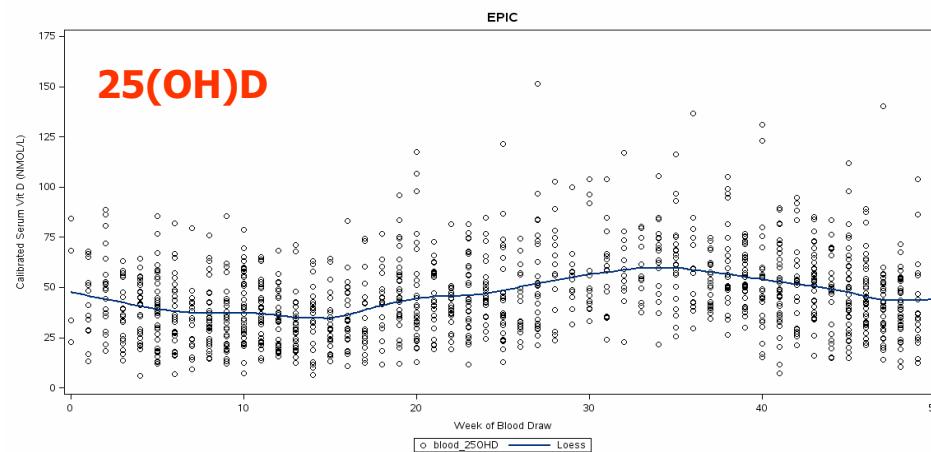
- Fasting – very important!
- Many exposures can change biomarker levels for short intervals
 - Smoking
 - Alcohol intake
 - Physical activity
- Important to be able to account for these factors in the study design or data analysis
- Other sources of variation...

When should sample be collected?



Kaaks et al,
JNCI 2005

Smith-Warner,
in progress



Where to collect

- Participant convenience and feasibility
 - Can sample be collected “on demand”?
 - Does timing need to be taken into account?
 - Ease of collection
- Rapidity of processing required
- Cost
- The ‘where’ can impact response / compliance

Collection Options

- Clinic or hospital
- Local phlebotomy laboratory
- Mobile van
- At home
- Mailed kits
- Workplace
- Charity walk/run
- And many more...

Ideal sample collection and processing

- Collect fasting samples; probably standardize timing of collection
- Collect with several different preservatives
- Process within 1 hour of collection
- Keep sample cold at all times
- Freeze sample immediately after processing
- Aliquot sample into many small volume tubes (or straws) for future use

Reality

- Keep sample cool
 - Challenge in rural areas/developing countries
 - Use ice pack / frozen water bottle
- Process quickly after collection
 - Participants cannot come to you
 - Sample cannot be collected on demand
 - Use overnight shipping – expensive

Reality

- Freeze immediately
 - Freezers are often offsite
 - Ship with dry ice (always include extra)
 - Freeze to a moderate temp before shipping
- Make many small aliquots
 - Freezer space may be limited
 - May not know what analysis will be done
 - Make "lab-ready" aliquots and larger aliquots that can be accessed later

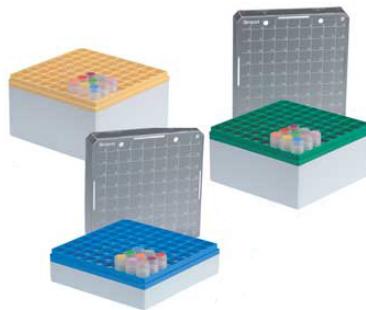
Pilot, pilot pilot!!

Sample storage

- Complex set of choices to maximize use of samples
 - Cryovials / labels
 - Aliquoting
 - Freezers
 - Data management
 - Safety
- Make decisions and set up protocols before collecting samples

Tubes and labeling

- Tube Type: Glass or plastic cryotube or cryostraw
- Plastic versus cardboard boxes
- Split sample from same person across freezers
- Storage of remaining sample (once initial aliquot used)
- Good data management is essential!!
- Label samples to minimize work to determine what they are in the future

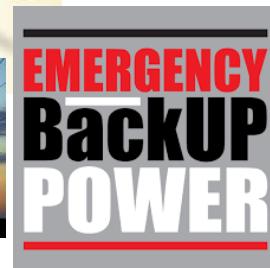


Tracking of samples

- Initial sample attributes
 - Sample type, volume, hemolyzed, contaminated, when collected, when processed, when frozen
- Whether aliquot present or removed
- Subsequent sample attributes, e.g.,
 - New volume(s) and volume remaining, new location(s), extra freeze thaw cycle,
- What labs it has been sent to / assay results

Storage considerations

- -4 degrees Celsius (generally not good)
- -20 degrees Celsius (okay for DNA)
- -70 or -80 degrees Celsius
- Liquid Nitrogen (-130° to –196° C)



Monitoring of samples

- 24-hours a day, 365 days a year!
- Detailed up-to-date instruction manuals
- Have back-up freezer(s) available if possible (or know where you can get loaners)
- Document any problems that could not be avoided

NPR Headline:

Thaw At Brain Bank Deals Setback To Autism Research



Collection, Processing, Storage: Summary (1)

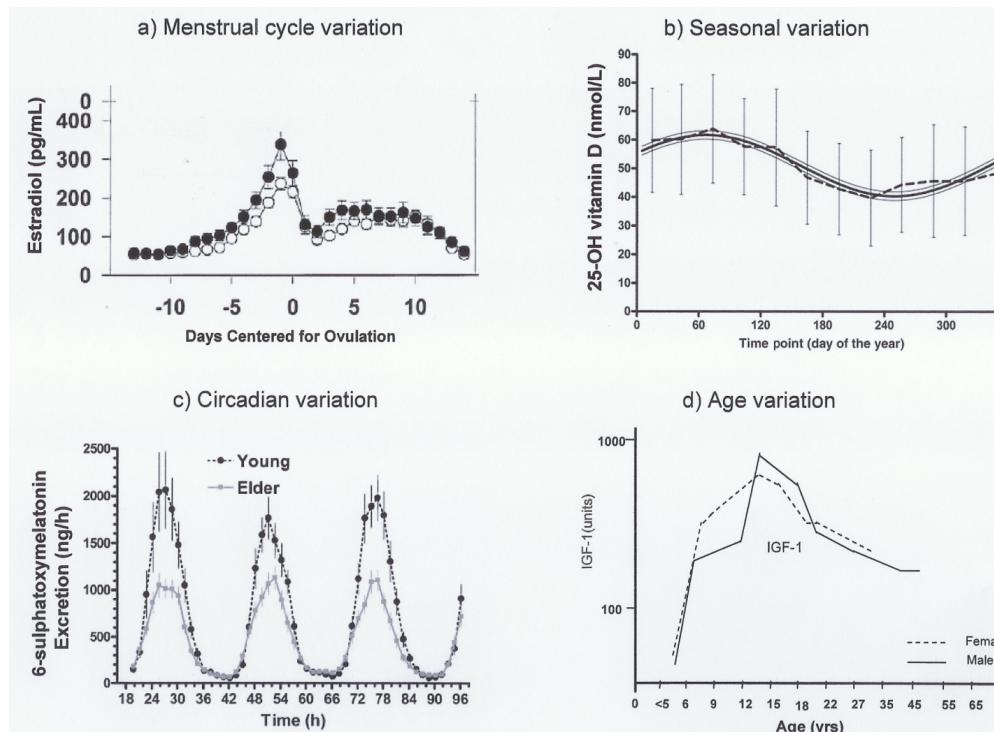
- Consider choices to make specimens most flexible for a number of assays
- Must consider cost vs. ideal methods
 - Liquid nitrogen freezer coldest, but can be more expensive/difficult to maintain than mechanical freezers
- Most studies are not able to do everything in most ideal manner; balance ideal with practical

Collection, Processing, Storage: Summary (2)

- Always talk with laboratory investigators prior to starting collection
- Always pilot test any non-standard methods
- Always budget enough money to do pilot testing

Measurement Error in Assays

Types of biologic variation, just to name a few...



Hankinson & Tworoger: Assessment of the Hormonal Milieu
In: Molecular Epidemiology: Principles and Practices

What is a “Batch”?

- A group of samples analyzed together on the same instrument, under a particular set of conditions
 - Assay technology and staff ability determines the batch/run size
- A lab should replicate relevant conditions across batches (e.g., reagent lot, technician)



Intra-assay error

- Assaying replicates of the same specimen will yield a distribution of results
- Intrinsic to the assay itself
- May be related to population being examined
 - E.g., sex hormones in postmenopausal women
- Reduce power by introducing random error
- Error can be quantified by the coefficient of variation
 - $CV=100\% * SD/mean$
 - CVs>20% are not good

Inter-assay error

- Occurs when there is an additional variability added due to changes in the assay between batches
 - Plays a particularly important role if assays are conducted over a long period of time
- Effect can be controlled somewhat at the study design and analysis phases and by communicating with the laboratory

Sample allocation

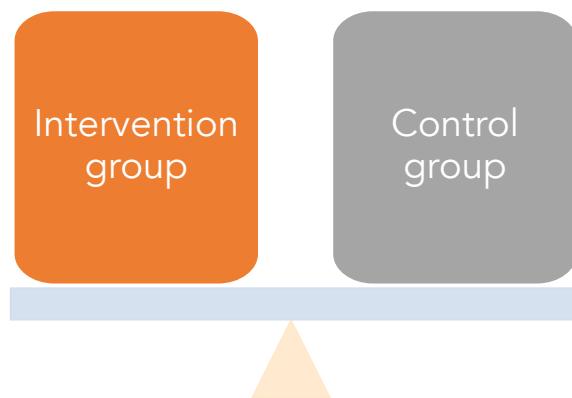
- Determining which samples are assayed in a batch
- Reduce the impact of inter-assay variability
- Approaches can be used that ensure
 - Effect estimates are not biased
 - Differences between comparison groups are not due to inter-assay variability

Principle #1: All samples from a related group assayed in the same batch

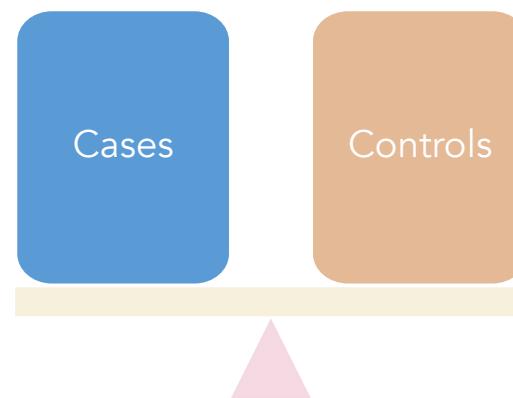
- Examples
 - Multiple samples from same participant
 - Matched case-control set
 - Other related group, e.g. a family set, twin pair, etc.
- Assures that differences between groups are not due to inter-assay variability

Principle #2: Comparison groups should be evenly balanced within assay batches

Randomized trial



Case-control study (non-matched or frequency matched)



Principle #3: a priori strata should, if possible, be balanced within assay batches

- Do this if particularly interested in the interaction term
- Examples:
 - Randomized trial stratify by gender
 - Case-control study of adiponectin and breast cancer should try to balance menopausal status

Batching samples: Method 1

- 1) Determine from lab the maximum batch size
- 2) You determine which samples go into the batch, without going over limit
- 3) Don't forget quality control samples



- Good for small batch size or many groups that need to be balanced

- Time consuming
- Can be difficult to implement on large scale

Batching samples: Method 2

- Put samples from a related set (e.g. case and matched controls) next to each other in the box, in random order and blinded
- Space sets so it is clear which samples need to be assayed together

	<ul style="list-style-type: none">- Good for large or variable batch sizes- Relatively simple		<ul style="list-style-type: none">- Cannot easily accommodate complex batching schemes- May not work well for samples from the same participant
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Sample Allocation examples

Batch for matched case-control (1:2) study

QC	QC	QC	Ca	Co	Co
Co	Ca	Co	Co	Co	Ca
Ca	Co	Co	QC	QC	QC
Co	Ca	Co	Co	Co	Ca

Batch for randomized trial (over time)

ID3.2	QC	ID1.1	ID2.1
QC	ID2.2	ID1.3	ID4.2
ID4.3	ID3.3	QC	ID3.1
ID1.2	ID2.3	ID4.1	QC

Active treatment group: IDs 1 & 4

Placebo group: IDs 2 & 3

Quality control samples

- ALWAYS, ALWAYS, ALWAYS include quality control (QC) samples (5-10% of samples)
- Obtain an independent and blinded evaluation of assay variability
- Lab is blinded, you have the key...
- Budget for QCs



QC samples

- Fluids: many replicates from fewer samples (e.g., pools)
- DNA: fewer replicates from many individuals
- Tissue: replicates from same tissue and/or multiple blocks from same individual
- Ideal: QCs should be like participant samples, but not the actual participant samples

Sending QC^s to the lab

- Mask the QC^s before sending to the lab, but make sure you have the key...
 - Use the same protocol, tubes, labels, etc.
 - Choose QC IDs similar to study IDs
- How many to include
 - 5-10% depending on CV in pilots
 - Somewhat depends on batch size
 - Remember to budget money for QC^s!!

Why it is critical to use BLINDED QCs

Lab	Within person CV		Bet. Person CV (%)	Observed RR for Specified True RR	
	Lab QC ^s (%)	Blinded QC ^s (%)		RR=1.5	RR=2.0
Lab 1	18	32	12	1.1	1.1
Lab 2	10	33	35	1.2	1.4
Lab 3	10	45	61	1.3	1.6
Lab 4	10	8	23	1.4	1.9

Hankinson SE, CEBP 1994

Real life example: What happened?

qctype	batch	assay A
N37	1	259.14
N37	1	214.96
N37	1	210.05
N37	1	202.11
N37	1	229.19
N37	1	213.08
N37	1	210.67
N37	2	620.66
N37	2	588.63
N37	2	547.56
N37	2	632.96
N37	3	216.10
N37	3	218.44
N37	3	227.62
N37	3	234.64
N37	3	205.06
N37	3	235.73

CV = 52%

qctype	batch	assay A
N38	1	187.39
N38	1	181.70
N38	1	172.49
N38	1	178.47
N38	1	195.70
N38	1	184.76
N38	1	183.88
N38	1	175.30
N38	1	176.73
N38	2	444.27
N38	2	460.27
N38	2	445.71
N38	3	170.20
N38	3	162.52
N38	3	187.41
N38	3	190.55
N38	3	171.08
N38	3	192.92

CV = 42%

qctype	batch	assay A
postqc2	1	222.41
postqc2	1	293.05
postqc2	1	246.91
postqc2	1	223.30
postqc2	1	282.77
postqc2	1	233.76
postqc2	1	250.77
postqc2	2	701.46
postqc2	2	494.00
postqc2	2	738.80
postqc2	2	619.43
postqc2	3	256.04
postqc2	3	230.69
postqc2	3	245.63
postqc2	3	226.63
postqc2	3	233.69
postqc2	3	241.99

CV = 53%

Investigation

- 1st Email to lab
 - Report of QC's: inter-batch CVs were 49%. Did anything strange happen in batch 2?
- Lab response
 - Internal controls were fine, no problems during assay
- 2nd Email to lab
 - Could this be a mathematical issue?
- Lab response
 - Yes, mathematical error when calculating values in batch 2

Corrected results CV=6%

Real life QC example

- Sent 800 case-control samples, with 90 QC samples. Assayed HDL, LDL, and total cholesterol.
- QCs were aliquoted from 18 different tubes containing the pool (e.g., large volumes were aliquoted into smaller volumes).
- The CVs were worse than expected (~15%), since these are standardized clinical assays

What happened?

QC vial	Cholesterol	HDL	LDL	HDL:Chol
1	157	43.1	87.2	0.27
1	158	43.4	87.8	0.27
1	174	46.6	96.6	0.27
1	205	55.9	115.4	0.27
1	217	59.2	121.6	0.27
2	152	41.7	85	0.27
2	169	44.9	91.4	0.27
2	231	61	128	0.26
3	138	37	74.4	0.27
3	158	42.8	88.4	0.27
3	161	43.9	90.5	0.27
3	196	51.8	108.5	0.26
3	233	61.6	130.3	0.26

Samples not mixed well before aliquoting

QC vial	Cholesterol	HDL	LDL	HDL:Chol
1	157	43.1	87.2	0.27
1	158	43.4	87.8	0.27
1	174	46.6	96.6	0.27
1	205	55.9	115.4	0.27
1	217	59.2	121.6	0.27
2	152	41.7	85	0.27
2	169	44.9	91.4	0.27
2	231	61	128	0.26
3	138	37	74.4	0.27
3	158	42.8	88.4	0.27
3	161	43.9	90.5	0.27
3	196	51.8	108.5	0.26
3	233	61.6	130.3	0.26

Altered vortexing
and aliquoting
protocol

Assay evolution

- Changes in technology may lead to different measurement modalities for a particular biomarker
- This may have largest impact on studies doing assays over long periods of time (e.g. nested case-control studies)
- Always check with laboratory when sending samples!

Implications – what to do

- Retest all samples
 - Cost
 - Feasibility
 - Specimen depletion / collection
 - Gain added value?
- Change assay midstream
 - Variation in absolute measurement
 - Comparison of old and new assay method

Other implications

- Comparing studies that use different assays or techniques
 - Can absolute levels be compared?
 - Is there heterogeneity by assay type?
- Opportunities for evaluation of new assays and technologies
 - Testing new assays & comparing to old

Assay evolution

- If assay changes over time, evaluate best course of action
 - Still use old assay
 - Use new assay for all samples
 - Use new assay for only new samples
- For new, “cutting-edge” assays, do a lot of pilots before embarking on large-scale studies.

Assay measurement error: summary

- All assays have error
- Work with the lab to reduce error
- Organize samples to reduce impact of error
- Include blinded QCs to assess error
- Assess effect of change in assay (pilot!)
- Evaluate how to reconcile old & new assay data

Overall summary

- Whether evaluating others' scientific work or proposing your own, think about ideal vs. reality:
 - Study design
 - Sample collection conditions
 - Choice of biomarker
 - Assay conditions

October 26:

Biomarkers of internal dose

- Ross et al., 1992. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*; 339(8799):943-46.
- Yuan et al., 2011. Urinary levels of the tobacco-specific carcinogen N'-nitrosonornicotine and its glucuronide are strongly associated with esophageal cancer risk in smokers. *Carcinogenesis*; 32(9):1366-1371.