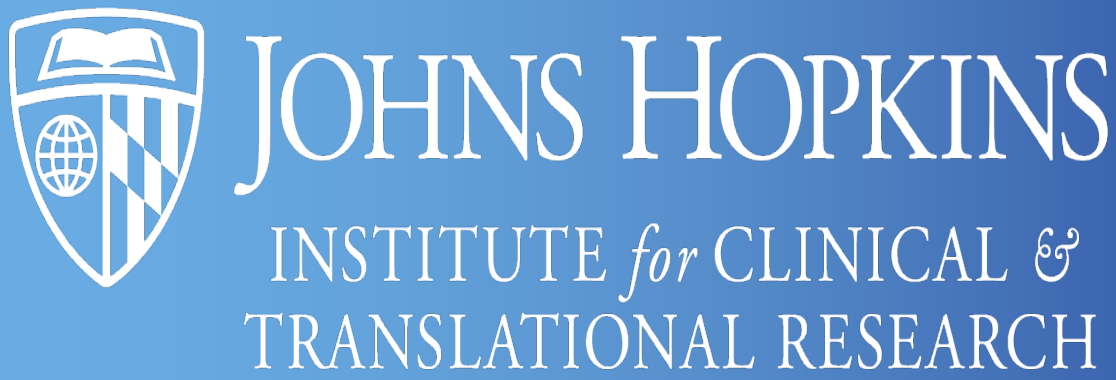
What the ICTR CRC lab can do for you and what you should know about tests & samples for clinical studies



ICTR Clinical Research Core Laboratory (ICTR CRC Lab)

But first…

- The average person can:
  - listen & process ~ 500 words per minute
  - read ~ 200 words per minute
  - speak ~ 100 words per minute

  - give her/his full attention for ~20 seconds

Objectives

Attendees will gain an understanding of:

- Identify Core Laboratory services of interest
- Understand basic lab work flow supporting clinical studies
- Specific types of specimen samples and their appropriate uses
- Sources of variability in biomarker measurements
- How to choose tests and assess the validity of their result

Outline

- An overview of the ICTR Core Lab
- How to request services
- Choosing samples
- Choosing tests

- Frequently asked questions

ICTR CRC Laboratory Purpose

Support clinical & translational research

Provide state of the art facilities and technical support for contemporary biochemical assays

Develop and validate new methods and biomarkers

JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

ICTR CRC Laboratory Purpose

Specialized immunoassays (>100 different)

On site (rapid) assays

- glucose/insulin for glucose clamps/IVGTTs provide "real-time feedback."
- creatinine, hemoglobin A1C, lipid panel

Facilitation of contemporary data management, reporting and interpretation with investigators.

Educational initiatives:

- Assay advising before study begins
- Assay validation

ICTR CRC Laboratory Services

Bone biomarkers
bone-specific alkaline phosphatase, creatinine, CrossLaps, deoxypyridinoline crosslinks, insulin-like growth factor-I, osteocalcin, parathyroid hormone, procollagen type I amino terminal peptides, 25(OH) vitamin D

Diabetes/obesity
adiponectin, c-peptide, ghrelin, gastric inhibitory peptide, glucagon-like protein, glucose, hemoglobin A1c, insulin, leptin, pro-insulin, resistin

Lipids
cholesterol, non-esterified free fatty acids, HDL, LDL, TGs, VLDL, Lp(a)

Hormones
ACTH, cortisol, DHEAS, estradiol, epinephrine, FSH, GH,
LH, norepinephrine, prolactin, SHBG, testosterone, TSH, T4

pro-inflammatory
C reactive protein, IL-6, INF-γ ,MMP-9, tPA/PAI-1, TNF-α

Adhesion molecules
sICAM, sVCAM, selectins, MCP-1

JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

Core Laboratory Service: Multiplex ELISAs

25 microliters = multiple simultaneous measurements

Cytokines
Any combination of:
IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNF-α

Chemokines
9-plex: Eotaxin, Eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1β, TARC

Cardiovascular
3-plex: CKMB, Myoglobin, Troponin I
4-plex: sICAM-3, E-Selectin, P-Selectin, Thrombomodulin
4-plex: CRP, sICAM-1, sVCAM-1, SAA

Growth Factors
4-plex: bFGF, VEGF, sFlt-1, PIGF

Hormones
3-plex: LH, FSH, Progesterone

Hypoxia
3-plex: EPO, IGFBP-1, VEGF

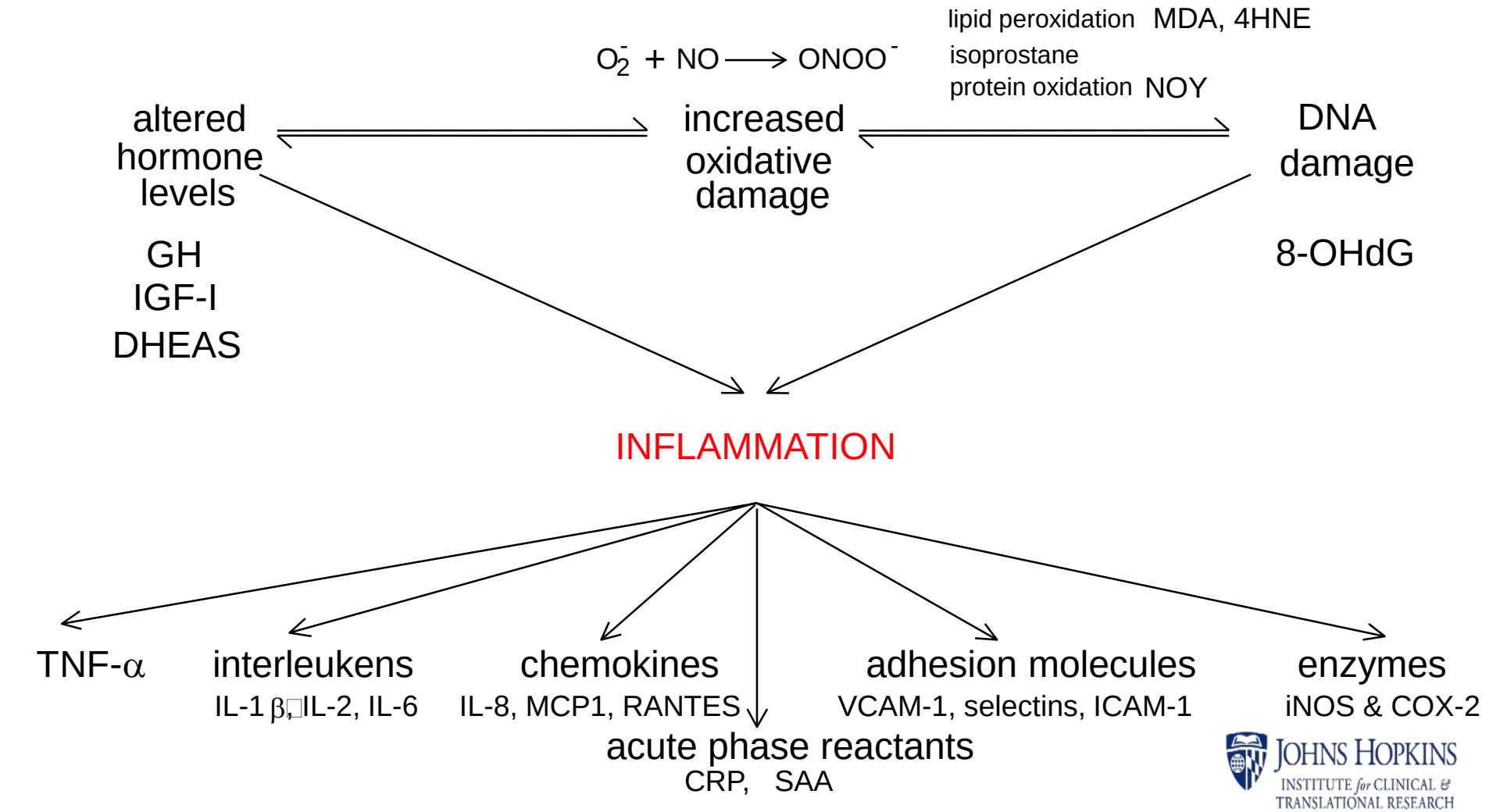
JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

genetic programming/ polymorphisms

diseases: hyperlipidemia, hypertension, hyperglycemia

environmental stressors

smoking, UV exposure, viruses



JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

Core Laboratory Workflow

Samples

- Samples from protocols logged in
  - To electronic sample log/study database
  - Freezer and assay tracking database
- Assays are performed on batches of samples
  - 36 to 40 samples/batch for ELISAs
  - 100 to 200 samples/batch for RIAs
- Ideally batch includes all samples from 1 protocol
  - Splitting of assays occurs to speed analysis
- Duplicate aliquots of samples are analyzed

Core Laboratory Workflow

- Each assay
  - Standard curve
  - Control samples
  - Unknowns
- Quality Control at the assay level
  - Historical 'graveyard' of standard curves
  - Tracking of control values (low, medium high)
  - Control values to calculate % coefficient of variance

    %CV = (standard deviation)/(mean) x 100
  - Unknown replicates

    Track %CVs to flag for repeats

    Mean values reported

Core Laboratory Workflow

- Results
  - Currently
    - Data sent electronically by agreement
  - Near Future
    - PI has web-based access to database containing results
  - Study Notebooks
    - Each study has a hard copy study notebook containing
      - Sample log, raw data, calculated data, standard curves, control values, replicate values, % CV values
      - PI's interested in obtaining replicate values or specific control values or %CVs for their assays are able to make copies from the study notebook.

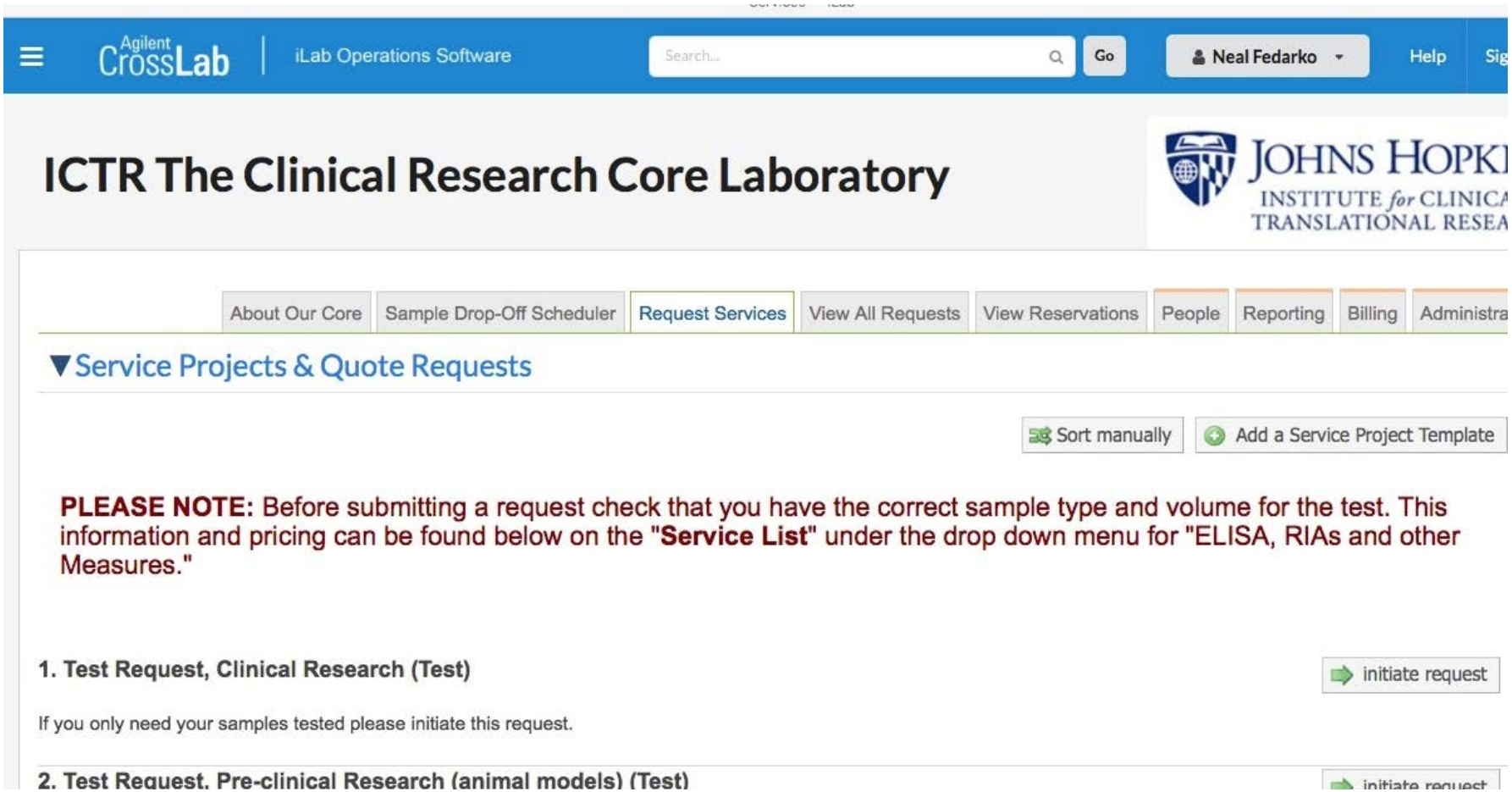
Core Laboratory Workflow

- Results
  - As assays are completed, PI's responsible for
    - authorizing repeat analyses
      - samples outside of linear range
      - samples with unacceptable &CVs
    - retrieving samples
    - or okaying lab tossing samples

Where are we?

- An overview of the GCRC Core Lab
- How to request services
- Choosing samples
- Choosing tests
- Frequently asked questions

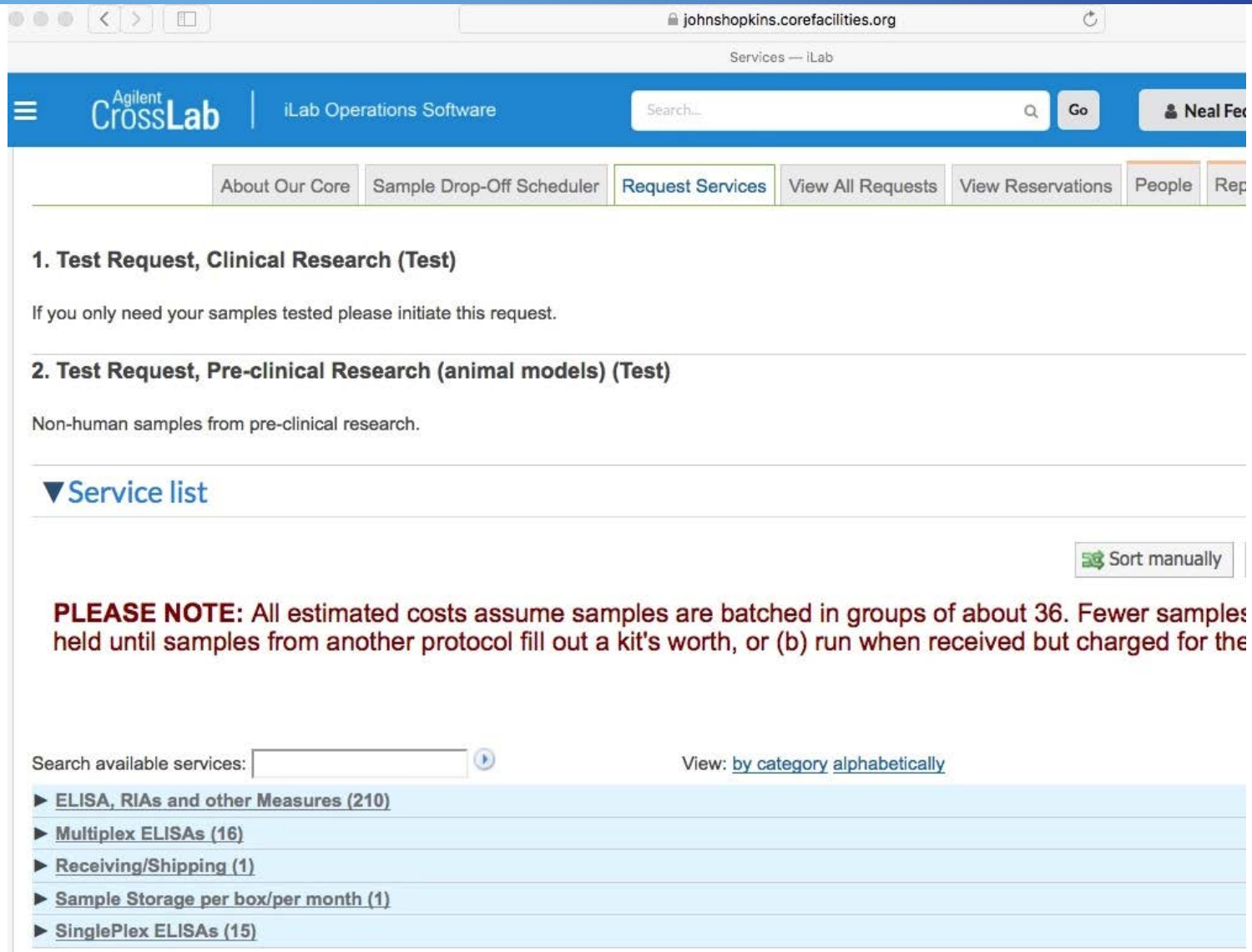
Requesting Services



JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

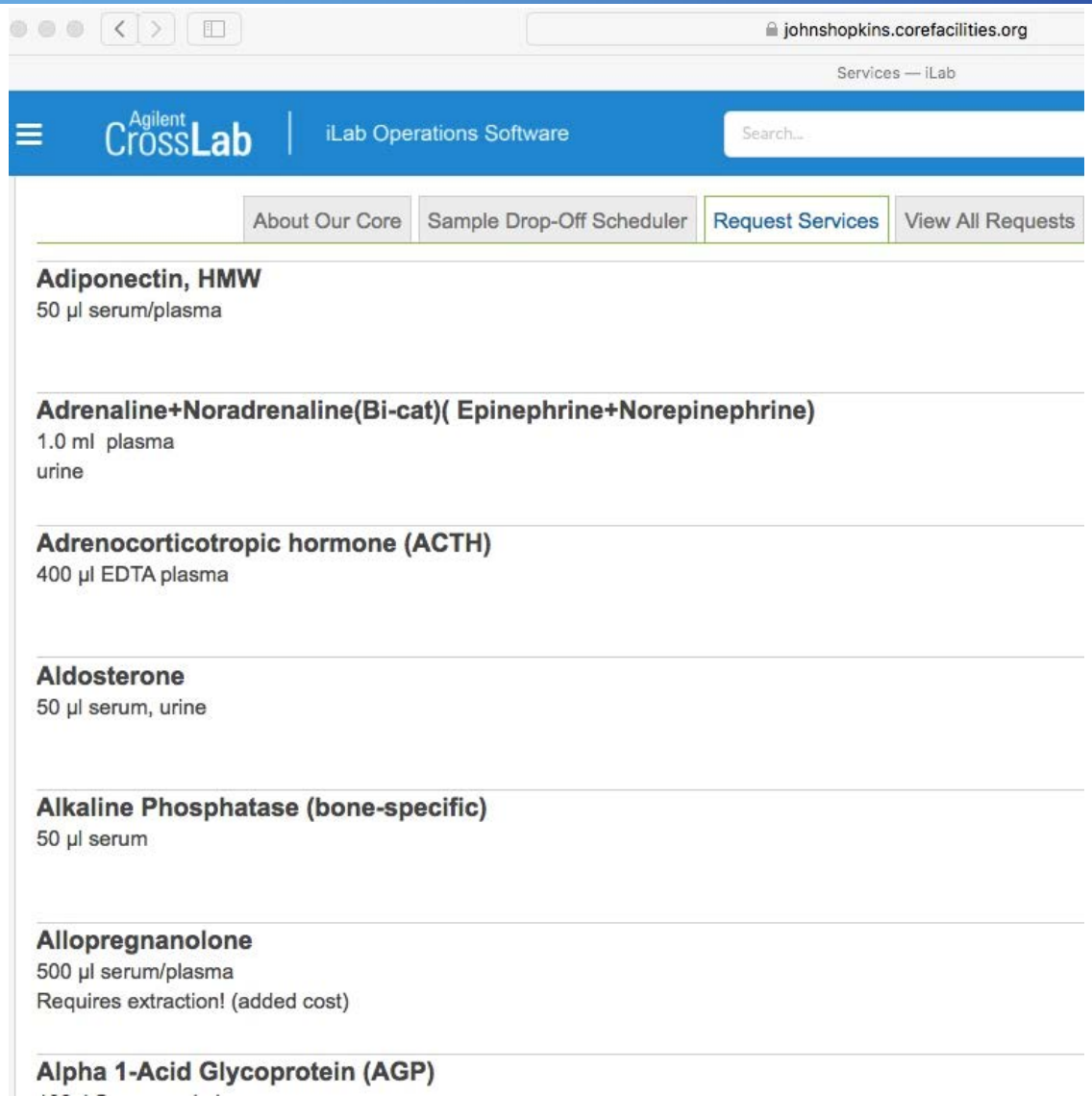
https://johnshopkins.corefacilities.org/service_center/show_external/3781

Requesting Services



JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

Requesting Services



Johns Hopkins
Institute for Clinical & Translational Research

Where are we?

- An overview of the GCRC Core Lab
- How to request services
- Choosing samples
- Choosing tests
- Frequently asked questions

Types of Specimens

Blood

Saliva

CSF

Bronchial lavage

Urine

DNA/RNA

Cells

Blood Collection

- Typical laboratory assays need 100 to 200 microliters (0.1 to 0.2 cc) or less
- The amount of blood collected per tube can be based on the number of tests and needs
- For tubes with additives, it is usually necessary to collect the entire quantity specified for the tube to maintain the blood additive ratio.

Blood Specimens

- Whole blood
- Serum
  - blood is allowed to clot, then centrifuged
- Plasma
  - Clotting is prevented, components separated
    - EDTA
    - heparin

Blood Specimens

Serum or plasma

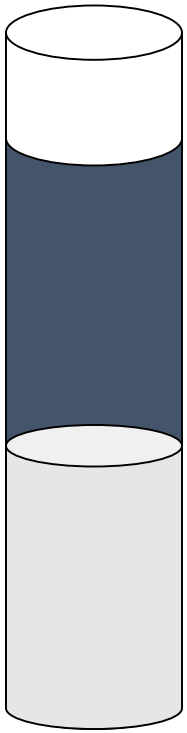
Liquid component

- 55% of total blood volume
  - 91% water
  - 7% blood proteins (fibrinogen, albumin, globulin, etc)
  - 2% nutrients (amino acids, sugars, lipids)
    - hormones (erythropoietin, insulin, etc)
    - electrolytes (sodium, potassium, calcium, etc)

Cellular components

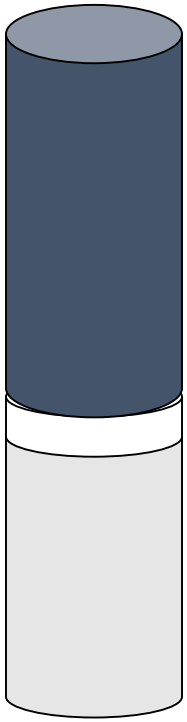
- 45% of total blood volume

Blood Specimens



serum

clot



plasma

buffy coat

red blood cells

white blood cells
platelets

Blood collection tubes

- Tubes are collected in a specific order based on perishability
- Investigator preference for tube collection takes precedence over a preset order
- **Serum** is collected from tubes without anticoagulant
- **Plasma** is collected in tubes with anticoagulant. Plasma contains clotting factors

Blood collection tubes

Specialized collection tubes have stabilizers and or inhibitors added.

Additives:

- Aprotinin
- DPP IV inhibitor
- Pepstatin
- p-Hydroxymercuribenzoate
- 1,10-Phenanthroline

Blood collection tubes

Special processing is sometimes required

Small molecular weight analytes often need to be separated from abundant larger molecular weight components in serum/plasma.

Peptides (eg. Ang II, ANP) require inhibitors present in the blood draw tube and extraction after generating plasma/serum.

Saliva Collection

- What does saliva measure?
- Why choose saliva?
- How does the collection work?

Saliva Collection: What?

- Cortisol – stress levels – used in early childhood development
- Testosterone – sexual and physical performance – behavior such as aggression, violence
- Progesterone – reproduction – hormone replacement therapy
- Secretory IgA – age, stress, exercise, disease related effects – immune function
- Cytokines

Saliva Collection: Why?

- Ease of collection – Minimal training is necessary and individuals can collect their own samples.
- Non-invasive method – It is much easier to recruit subjects for studies that utilize saliva.
- Cost effective – Supplies for collecting samples are much less expensive.
- Accuracy – Saliva levels are highly correlated with serum levels.

Saliva Collection: Tools

- Equipment Needs
  - Saliva collection tool
  - Cooler or dry ice mail envelope
  - Cooling packets or dry ice
  - Storage Facility (refrigerator freezer)
- Collection Protocol
- Timer

Saliva: cortisol

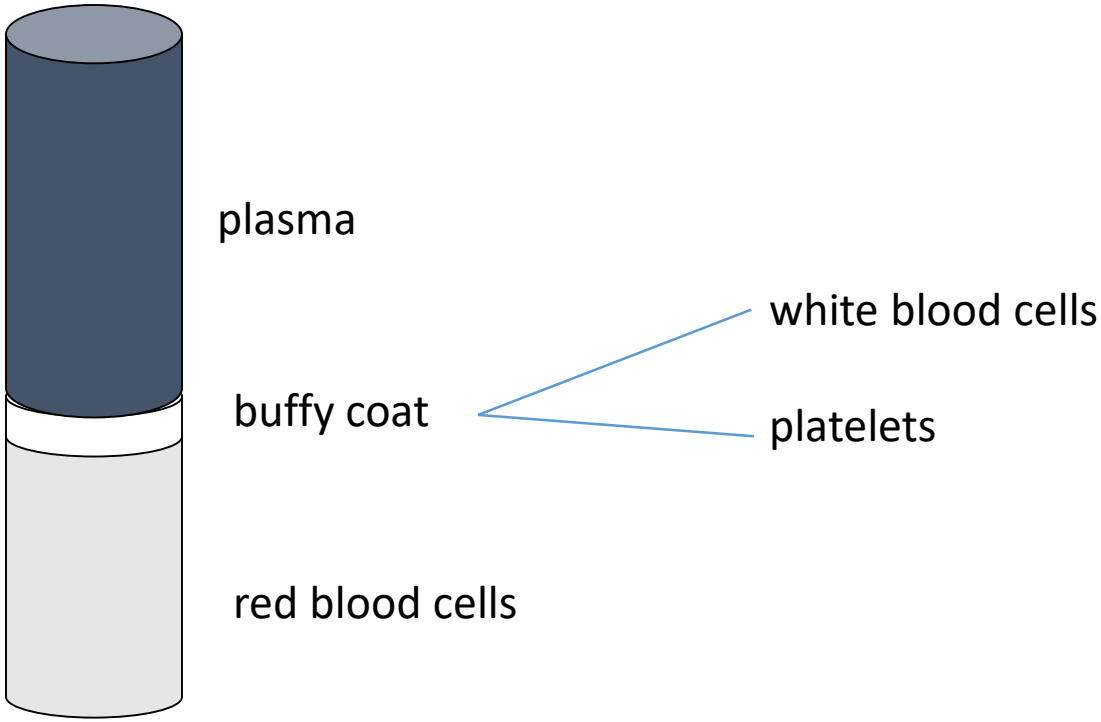
Cortisol in saliva: sample collection

- Timing of collection
  - Thorough understanding of diurnal cycle
  - Collection at standardized times (23:00)
- Contamination issues
  - No meals prior to sample (1 hour)
  - No alcohol during 1 day (interferes)
  - No dairy products (bovine hormones)
  - No acidic or high sugar foods (low pH)
  - No blood contamination!!!
- No saliva stimulants
  - ø Chewing gum
  - ø Lemon drops, sugar, etc.

Urine

- **Collection based on times**
- **Single voids**
  - Qualitative values of urine components
    - Urine HcG (highest in first morning void)
    - Urine Toxicology screen
    - Safety voids for protein, glucose, blood WBC etc.
- **Timed voids, most commonly 24 hour urines**
- **Types of collections**
    - Plain
    - Acid (contains 25-40cc of 6M Hydrochloric Acid which can cause chemical burns)
    - Acid (contains boric acid tablets)
    - Split 50/50 acid and plain

Cells, DNA/RNA collection



Cells

Options:

- Isolate buffy coat
- Isolate by adherent versus non-adherent culture
- Enrich by selection with markers (eg. CD40)
    - magnetically tagged Abs + cells
    - column in magnetic field to retain specific cells
    - remove magnetic field & elute enriched cells
- store in liquid N_2

DNA

Options:

- Isolate from whole blood
  - blood can be fresh or frozen
  - simple extraction procedure (“DNAzol”)
- Isolate from cells
  - leukocytes
- Isolate from buccal swab

DNA

“The highest quantity of DNA remained in samples stored at -80°C, regardless of storage additives, and those dried at room temperature in the presence of trehalose. Surprisingly, DNA quality was best preserved in the presence of trehalose, either dried or at -80°C; significant quality loss occurred with -20°C and +4°C storage.”

Smith, S. (2005) Optimal storage conditions for highly dilute DNA samples: A role for Trehalose as a preserving agent. Journal of Forensic Sciences, **50**(5)

RNA

Options:

- Isolate from whole blood
    - blood can be fresh or frozen
    - simple extraction procedure (“RNAzol”, Tri-reagent BD))
- Isolate from cells
- Store at -80

Where are we?

- An overview of the GCRC Core Lab
- How to request services
- Choosing samples
- Choosing tests
- Frequently asked questions

JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

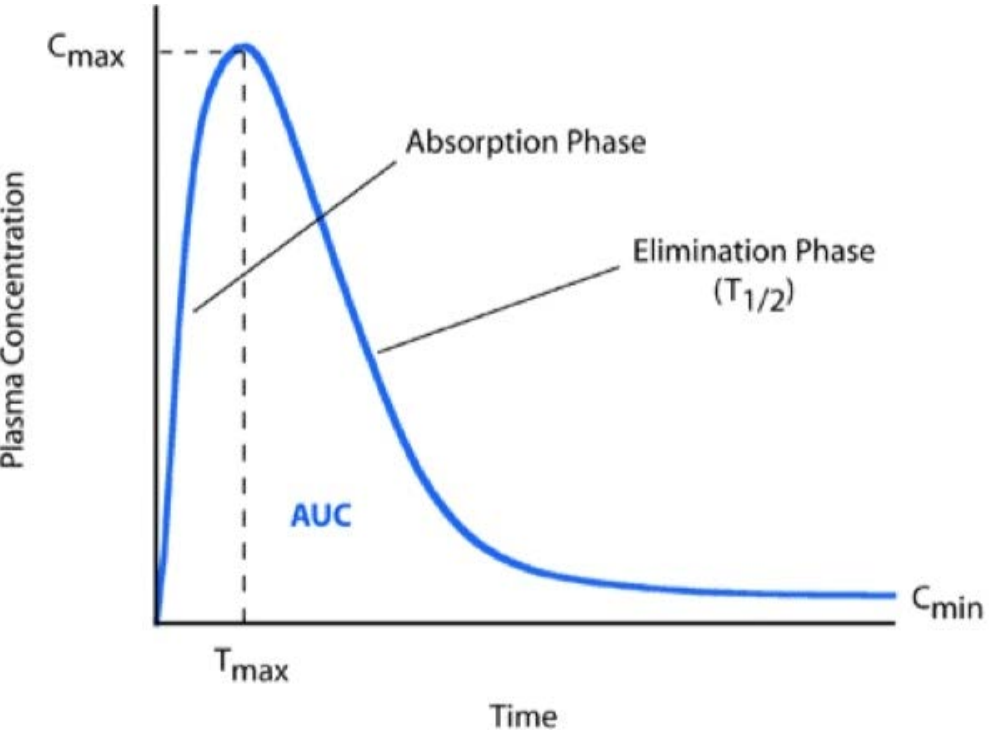
Types of Laboratory Data

- Qualitative Data
  - Data which is interpreted in nominal (yes or no) answers
  - Typical of safety data and certain stimulation tests
  - Certainty of data point around cut-off value
- Quantitative Data
  - Data points which have numerical values and can be compared to other data points for meaningful evaluation
  - Typical for data collection in clinical studies
  - Certainty of absolute data value

Single Data Points

- Single data points are those in which a single datapoint is used as the outcome
- Examples include:
  - 24 hour urine Calcium or Urine Free Cortisol
  - Urine Human chorionic gonadotropin (Hcg)
  - A bone density (dexa scan)
  - CBC to evaluate WBC count before preceding with a study

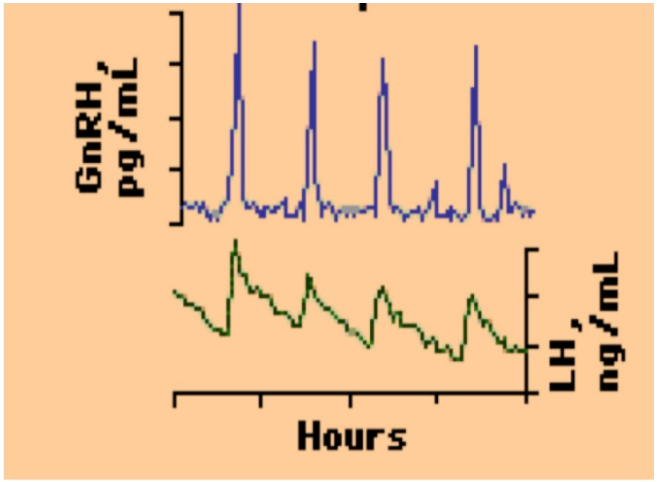
Multiple Data Points Pharmacokinetic (PK) studies



Typically used to evaluate uptake, bioavailability and elimination of drugs from the body.

PK studies can be used to compare differences between groups of individuals or to check drug interactions.

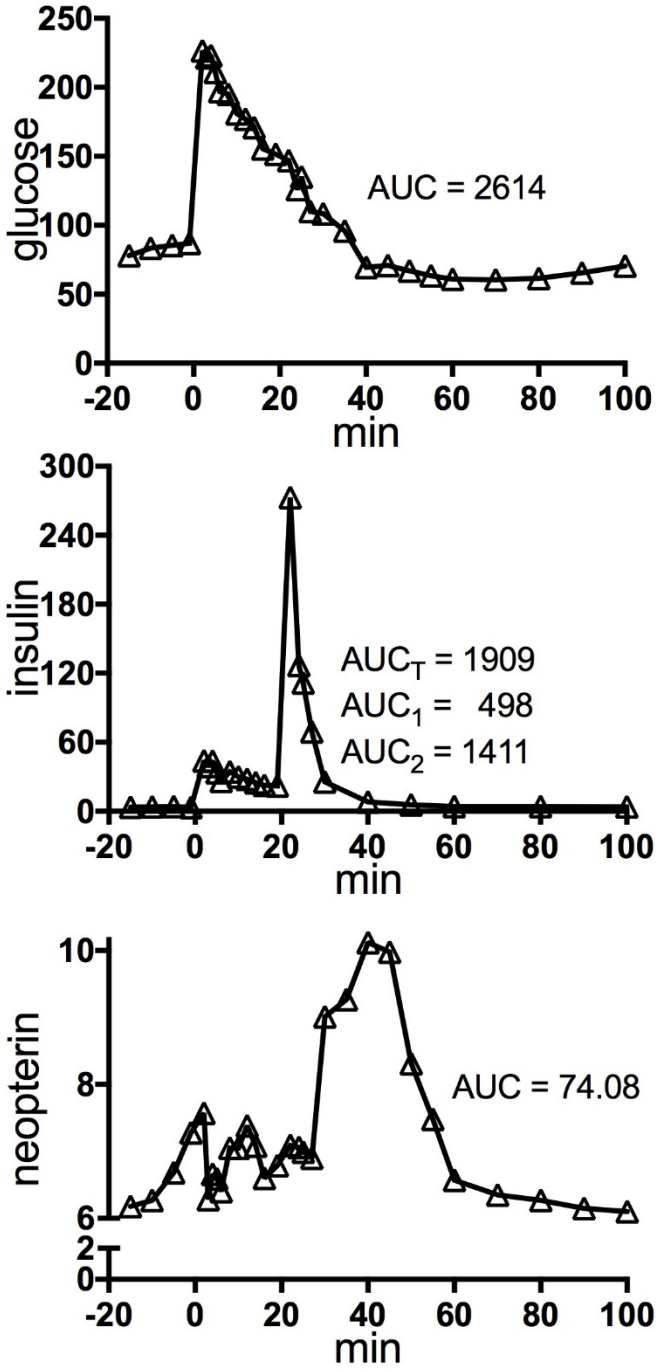
Multiple Data Points Physiologic studies



- Time periods may vary from
  - Minutes
  - Hours
  - Days
  - Months
  - Years
- Precision of data collection dependent on:
  - Amplitude of the pulse (variation from baseline)
  - Frequency of the pulse (how often the pulse occurs)

Stimulation Tests

- These tests are typically done to compare levels post administration of a stimulus to the pre-dose level
- The post dose points might be single points (qualitative test) or multiple points (quantitative test). Examples include:
  - Oral glucose tolerance test
  - Cosyntropin test for adrenal function



Variability

- Pre-analytical
- Analytical
- Biological variation
  - Within-subject (intra-individual)
  - Between-subject (inter-individual)

Pre-analytical variability

- Age
  - highest values = birth & infancy
  - increase again at puberty
  - lowest during adulthood

Pre-analytical variability

Sex

- gender differences at puberty
  - slightly **elevated** in ♀
- significant differences peri- & postmenopausal
  - **values** decreased in women

Pre-analytical variability

- Seasonal
  - elevated during winter, decreased during summer
  - Associated with vitamin D metabolites

Pre-analytical variability

- Physiology
  - Fractures
    - increased values up to 1 year
  - Pregnancy & lactation
    - peak at term and beginning months of lactation
  - Diseases
    - Hyperparathyroidism,
    - Hyperthyroidism
    - Cancers
    - Anorexia
  - Medication
    - corticosteroids

Pre-analytical variability

circadian & among day variation

Biomarker	circadian*	among day
NTX, urine	40%	18%
NTX, serum	65%	-
CTX, serum	-	6.9%
Pyd, urine	73%	17%
Dpd, urine	70%	17%

*nadir/peak

Pre-analytical variability

Diurnal variation

Serum Cortisol at 8:00 and 20:00.

- Avoid stress!

– Normal:-

- 08:00h - 10:00h 10 - 22 µg/dl
- 20:00h - 24:00h < 10 µg/dl

or 50% of 08:00h value

– Cushings:-

- Loss of diurnal variation.

JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

Stability

PINP, serum

> 48 h at 23 - 25 C
7 days at 2 - 8 C
6 months at -20 to -80 C
< 5 freeze/thaws

Pyd & DPD, urine

< 24 h at 23 - 25 C
7 days at 2 - 8 C
>1 year at -80 C
< 2 freeze/thaws

NTX & bALP, serum

≤ 72 h at 23 - 25 C
7 days at 2 - 8 C
>1 year at -80 C
< 5 freeze/thaws

OC, serum

< 8 h at 23 - 25 C
1 day at 2 - 8 C
>1 year at -80 C
no freeze/thaws

Clin Chem 46(8):1200-1202, 2000 & ICTR CRU Lab, unpublished

Stability with freeze/thaw cycles

Cytokines are perturbed by freeze-thaws cycles

sTNFR1 levels are stable for up to 3 cycles

Adiponectin is stable for up to 2 cycles

Resistin levels are stable for up to 5 cycles

Leptin levels are not altered by up to 6 cycles

Some biomarkers are unaffected

Analytical variability

(assay variation*)

Biomarker	%CV intra	%CV inter
Bone ALP, IRMA serum	5.49	5.83
CTX, EIA seurm	2.90	8.19
DPD, EIA urine	6.44	7.79
OC, IRMA serum	2.97	6.30
PICP, RIA serum	2.60	5.18
PICP, RIA serum	4.14	2.74
NTX, EIA urine	4.56	8.83
NTX, EIA serum	3.10	6.23

ICTR CRC lab

Analytical variability
(inter laboratory variation*)

Biomarker	%CV
DPD, total urine, HPLC	28.0
DPD, free urine, EIA	12.0
Bone ALP, IRMA	20.5
Bone ALP, EIA	18.5
OC, EIA	27.5
OC, RIA	69.5
NTX, EIA	39.0

*among 13 US labs

Where are we?

- An overview of the GCRC Core Lab
- How to request services
- Choosing samples
- Choosing tests
- Frequently asked questions

JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH

Frequently Asked Questions

- I can't find the biomarker I want measured listed in iLab.

  can you measure it?

- If you can't find an analyte/biomarker contact the lab. Not every analyte is listed in iLab and new ones are added fairly frequently.

Frequently Asked Questions

- What happens if my reported sample values are too low (or high)?
  - factors effecting samples values
    - extremes of age (very young or old)
    - pathology (abnormal range)
    - treatments (eg anti-inflammatory)
  - sample re-analyzed at different dilution
  - sample re-analyzed with different sensitivity kit

  - Investigator needs to approve re-analysis

Frequently Asked Questions

- How does the lab determine which assay to use?
  - required sensitivity:

RIA	µg to ng
IRMA	µg to ng
ELISA	µg to ng
EIA	ng to pg
CLIA	ng to pg
FLIA	pg to fg

Frequently Asked Questions

- How does the lab determine which assay to use?
  - required sensitivity
  - reproducibility
    - low coefficient of variance (%CV)
    - stable control values
  - robustness
    - good range of linearity
    - high precision data
    - assay neither too technically difficult or time consuming
  - results
    - agree with existing literature

Frequently Asked Questions

- What type of sample (serum or plasma) should be used for blood measures?
  - factors determining choice
    - test specific
    - plasma
      - epinephrine & norepinephrine
      - fibroblast growth factor-23
      - homocysteine
      - myeloperoxidase
      - PAI-1, tPA, vWF

Frequently Asked Questions

- What type of sample (serum or plasma) should be used for blood measures?
  - factors determining choice
    - test specific
    - plasma
      - small polypeptides requiring extraction

        ANG II, ANP, BNP, GLP-1, GIP, NPY, obestatin, PYY

Frequently Asked Questions

- What type of sample (serum or plasma) should be used for blood measures?
  - factors determining choice
    - test specific
    - serum
      - bone metabolism markers

        procollagen amino- & carboxy-terminal propeptides, CrossLaps, dexypyridinoline crosslinks, ICTP, etc.

Frequently Asked Questions

- What type of sample (serum or plasma) should be used for blood measures?
  - factors determining choice
    - existing literature preference
      - eg : plasma cytokine values for cardiology
      - check the literature in <u>your</u> field!
    - what samples are available
      - historical samples
      - ease of collection
        processing
        storage

Frequently Asked Questions

- How do you maintain QC/QA?
  - each test includes control samples
    - values within acceptable limits
    - %CVs within acceptable limits
  - standard curve "graveyards"
    - ED 80, 50 and 20 values
  - equipment linearity check
  - technician proficiency testing
  - GLP and CLIA compliant

Contacts

- Lab Director

Neal Fedarko, 410-550-2632, ndarko@jhmi.edu

- Lab Manager
- Raisa Gelman, (410-550-1958, rgelman2@jhmi.edu

JOHNS HOPKINS
INSTITUTE for CLINICAL & TRANSLATIONAL RESEARCH