

University of South Florida
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Substrate and Substance in Biobehavioral Research



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What affects results in biomarker measurement?

- Sample used for biological analysis
- Collection methods
- Analysis methods

The sample matters

- Blood (plasma, serum), urine, CSF, saliva, EBC, etc
- Not all biological fluids are equal!
 - Blood differs from urine
 - Blood differs from saliva
 - Plasma differs from serum
- Different fluid compartments have different composition
 - Transport between compartments
 - Differences in biological needs of compartments
- We know this in clinical measures, but sometimes forget it in research!

Collection method matters

- How was the sample collected?
 - Technique
 - phlebotomy- hemolysis
 - Saliva- stimulation and method
 - Additives (and anticoagulants)
 - pH
 - Container/ collection device
 - Differential protein binding
- How was it stored?
 - Temperature
 - Freezing speed
 - Freeze-thaw damage

Analytic method matters

- In the OLD days...
 - We made our own assays!
 - Assumed that there was variability
 - Interpretation more qualitative
- The new-fangled way
 - Buy a commercial kit
 - Assume no problems with variability
 - Interpretation more quantitative

Analytic method matters

- Multiplex multi-problems (thanks to Maureen Groer!)
- Breen (2011)
 - 6 Labs, 4 multiplex assay kits
 - “No single multiplex panel detected all cytokines, and there were highly significant differences ($p < 0.001$) between laboratories and/or lots with all kits.”
- Ellington (2010)
 - Plate-to-plate variability accounted for 24.4% of the analyte total variability

The Matrix

- “the sum of effects of all components (both qualitative and quantitative) in a system, with the exception of the analyte to be measured”
- differences in
 - pH,
 - proteolytic enzymes
 - viscosity
 - assay diluents and buffers
 - dilution factors
- determination of sample incubation times, reduction of cross-reactivity between assays, and nonspecific protein binding

Controls

- Most commercial kits calibrated for serum or cell culture
 - Other samples have differences in protein concentrations, pH, osmolarity
- Variability in assay manufacturing processes
- Nonspecific binding, background signal, decreased assay sensitivity

Compare with caution

- Methods often not well described
- Difficult to compare absolute values
- Quality control protocols
 - range of linearity
 - analytical specificity
 - recovery
 - limits of detection and quantification
 - reference method
- Most biobehavioral assays are not “clinical” type tests

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Thank You!

