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Applied Translational Medicine

Do you have a Validated Biomarker for this Compound?

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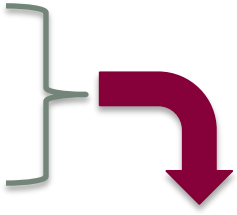
Do you have a validated biomarker for this compound? Questions that need to be answered

- Is this for a small molecule or a large molecule?
- What is the species? Is this the right biomarker for this species?
- What is the intra-subject and inter-subject variability?
- **Is this data going to be used for a PK/PD plot or for a statistical comparison?**
- Is this an endogenous biomarker? Will this biomarker be present in the matrix? Do different disease states affect the concentration of this biomarker?
- **Is this biomarker present in the environment?**
- Is reference material available for this biomarker?
- **How much are you are willing to spend for this assay?**

Presentation Outline

- Role of Biomarkers in MRTP development
- Fit-for-purpose Bioanalytical Method Validation
  - The continuum of biomarker validation
  - Examples where improvements to the method can result in decreasing the clinical study size
    - Sample Collection
    - Assay sensitivity
    - Assay precision



- Conclusion: Do you have a validated biomarker for this assay that will reduce clinical costs?
- The ability of the biomarker assay to affect the clinical study is exciting!

Modified Risk Tobacco Product (MRTP) - FDA Guidance

- Exposure Modification Order (Section 911(g)(2)) requires
  - Scientific evidence of substantial overall reductions in exposure to the harmful substance(s)
  - The available scientific evidence demonstrates that a measurable and substantial reduction in morbidity/mortality among individual users is reasonably likely in subsequent studies
  - The order would be appropriate to promote the public health

Source: FDA Draft Guidance: Modified Risk Tobacco Product Applications

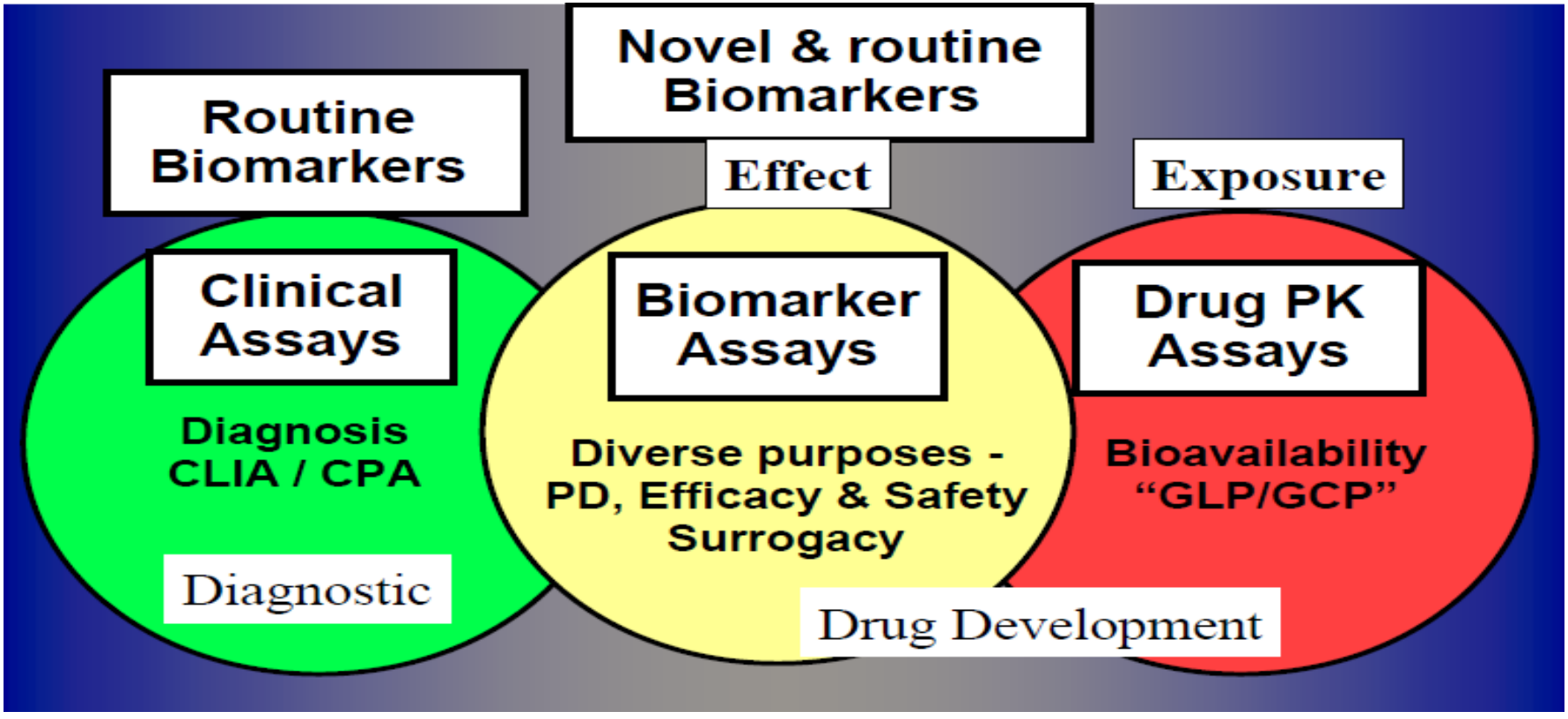
Challenges to Establishing Reduced Harm using section 911(g)(2)

- MRTP development requires faster and more controllable methods to assess smoking related diseases other than epidemiological studies
- Biomarkers offer an alternative and cost-effective approach for evaluation of potential harm reduction from MRTPs during product development
- The profiles of biomarkers may be used to understand biological events from smoke inhalation to disease manifestation
- Endpoint is the biological effect in response to smoking as opposed to disease manifestation

How SMART is your Biomarker?

- As suggested by Shehabi and colleagues a SMART biomarker is:
  - Sensitive (and selective)
  - Measurable (with high precision)
  - Available (affordable and safely attainable)
  - Responsive (and reproducible)
  - Timely (to allow decision making)
- And to be SMARTER one adds Evaluate (validate) and Re-evaluate (revalidate)

Fit for Purpose Biomarker Validation



When biomarker data will be used to support a regulatory action, such as the pivotal determination of safety and/or effectiveness or to support labeled dosing instructions, the assay should be fully validated in a bioanalytical lab.
- FDA BMV Guidance 2013

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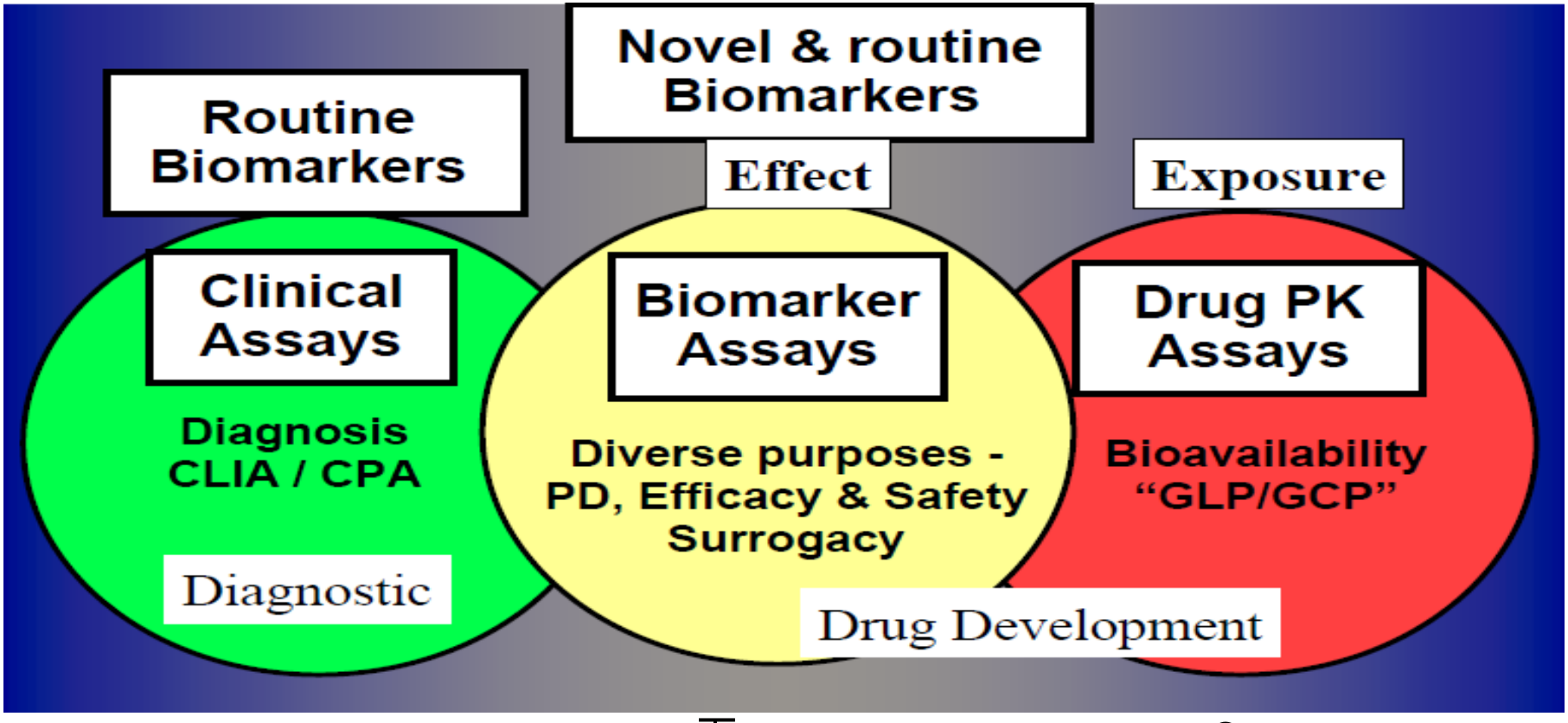
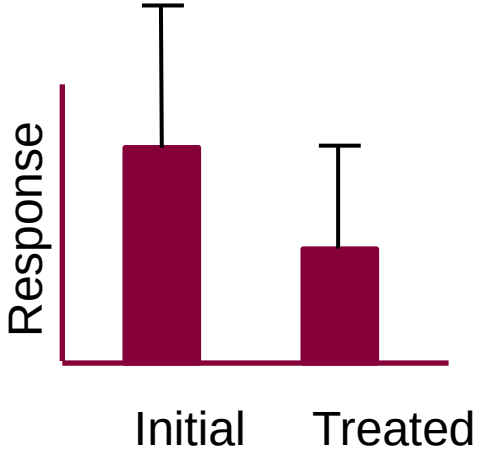
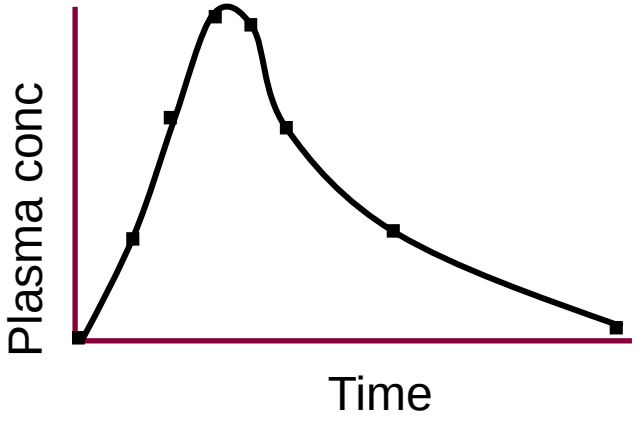
Fit for Purpose Biomarker Validation
What is the purpose for each assay?



Is the value normal or abnormal?





Must use the right assay for the right purpose:

- Example (CDER)
  - Company was developing a diabetes drug
  - Conducted a clinical study – GOAL: demonstrate a statistical decrease in blood glucose levels
  - Used a diagnostic clinical chemistry assay because it was cheaper
  - FDA rejected the study
  - Study was rerun and samples were analyzed using a bioanalytical biomarker assay
  - Study was accepted by the FDA
- Lesson learned for section 911(g)(2) filing?

iPF_{2a}-III Oxidative Stress Biomarker

- Identify the “validated method” you would like to use to evaluate tobacco smoker exposure risk as a primary clinical end point.

Study	Detection Method	Concentration of Healthy Normals (ng/mg creatinine)
Sircar et al.	LC-MS	0.36 (0.13)
Ohashi. et al.	LC-MS	0.43 (0.06)
Mori et al.	GC-MS	1.14 (0.02)
Lee et al.	GC-MS	0.50 (0.40)
Tsikas et al.	GC-MS	0.29 (0.04)
Liang et al.	LC-MS/MS	0.25 (0.15)

Bioanalytical Tobacco Assay Method Validation

- Bioanalytical Guidance – assay must be:
  - Appropriately collected ✓
  - Selective
  - Sensitive ✓
  - Precise ✓
  - Stable
    - Sample collection and handling
    - Freezer (-20°C or -80°C)
    - Freeze/Thaw
    - Benchtop
    - Pre-extraction
    - Post-extraction
  - DOCUMENTED !

Method Validation – Sample Collection

- Stability to light
  - Mercapturic Acids and some Tobacco Specific Nitrosamines are light sensitive in urine.
  - Must collect and transport the urine samples in light impenetrable tubes
- Minimize environmental contamination:
  - o-toluidine, an aromatic amine, was shown to leach from certain urine collection containers (4X > LLOQ) and other types of plastic ware.
  - Nicotine is ubiquitous (tube contamination 20X > LLOQ) - Methanol rinsed tubes, pipettes, etc. eliminates contamination.

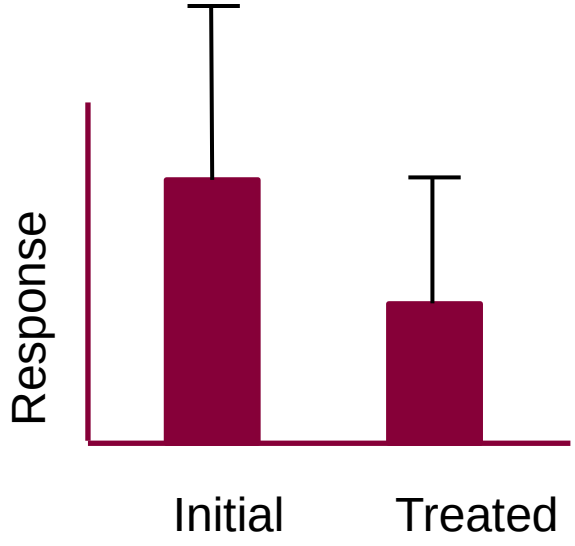
Method Validation – Sample Collection
COST SAVINGS

- If you don’t collect the samples properly to stabilize them and to eliminate contamination then don’t bother to run the study.

- Proper collection and sample handling requires the preparation of a detailed sample handling manual.

Method Validation – Sensitivity
EXAMPLES: NNN & 3-OH-B[a]P

- Old LLOQ (most sensitive)
  - NNN = 0.70 pg/ml
  - 3-OH-B[a]P = 50 fg/ml
- Old assay results (% of clinical samples that were < LLOQ):
  - NNN: >20%
  - 3-OH-B[a]P: > 40%
- Solution: purchased an AB Sciex 6500 LC-MS/MS and developed a new assay:
  - NNN LLOQ = 0.20 pg/ml
  - 3-OH B[a]P = 25 fg/ml
  - Dramatic decrease # samples < LLOQ



If a sample is < LLOQ then that subject cannot be included in the statistics

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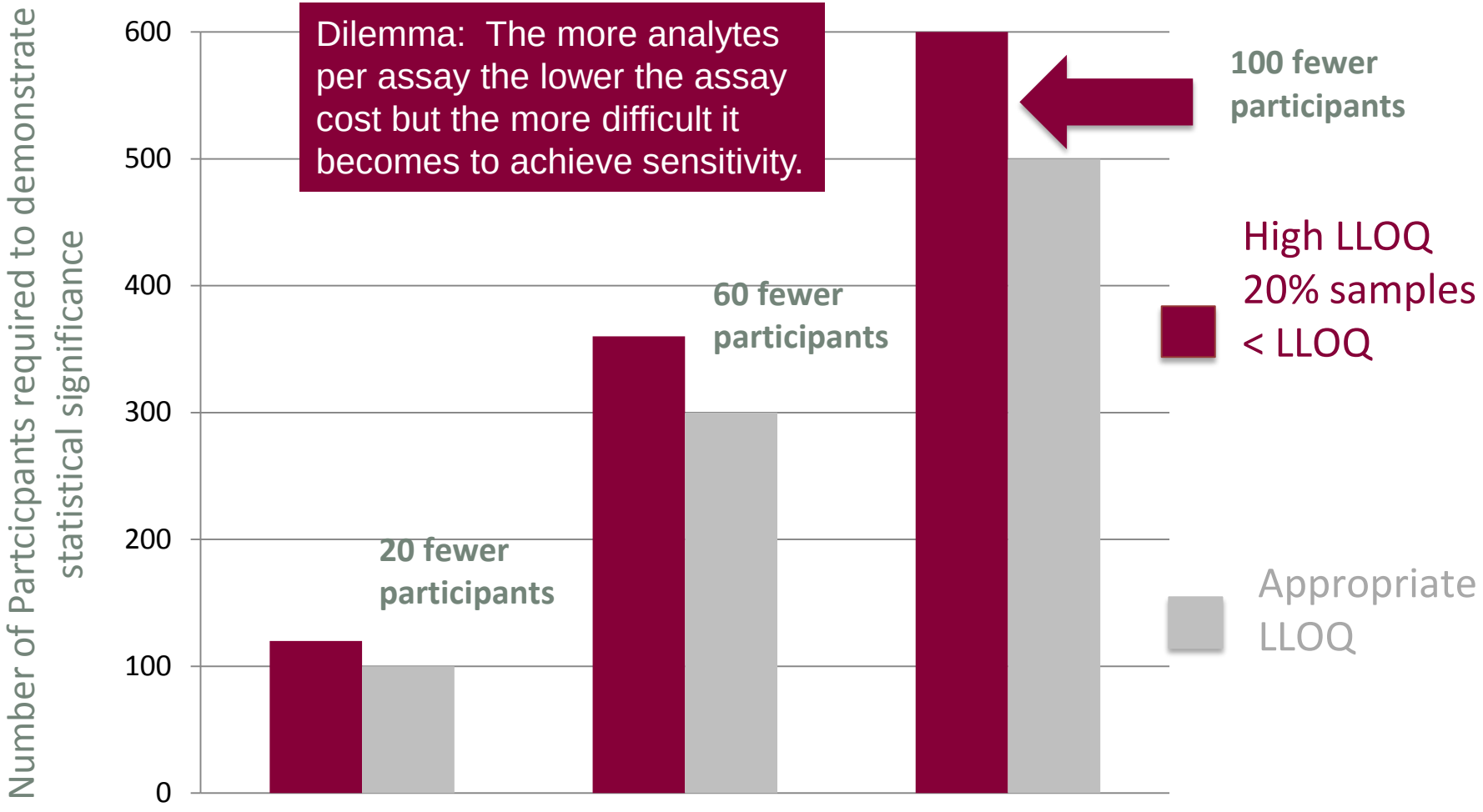
Method Validation – Sensitivity
COST SAVINGS: Able to dose fewer participants



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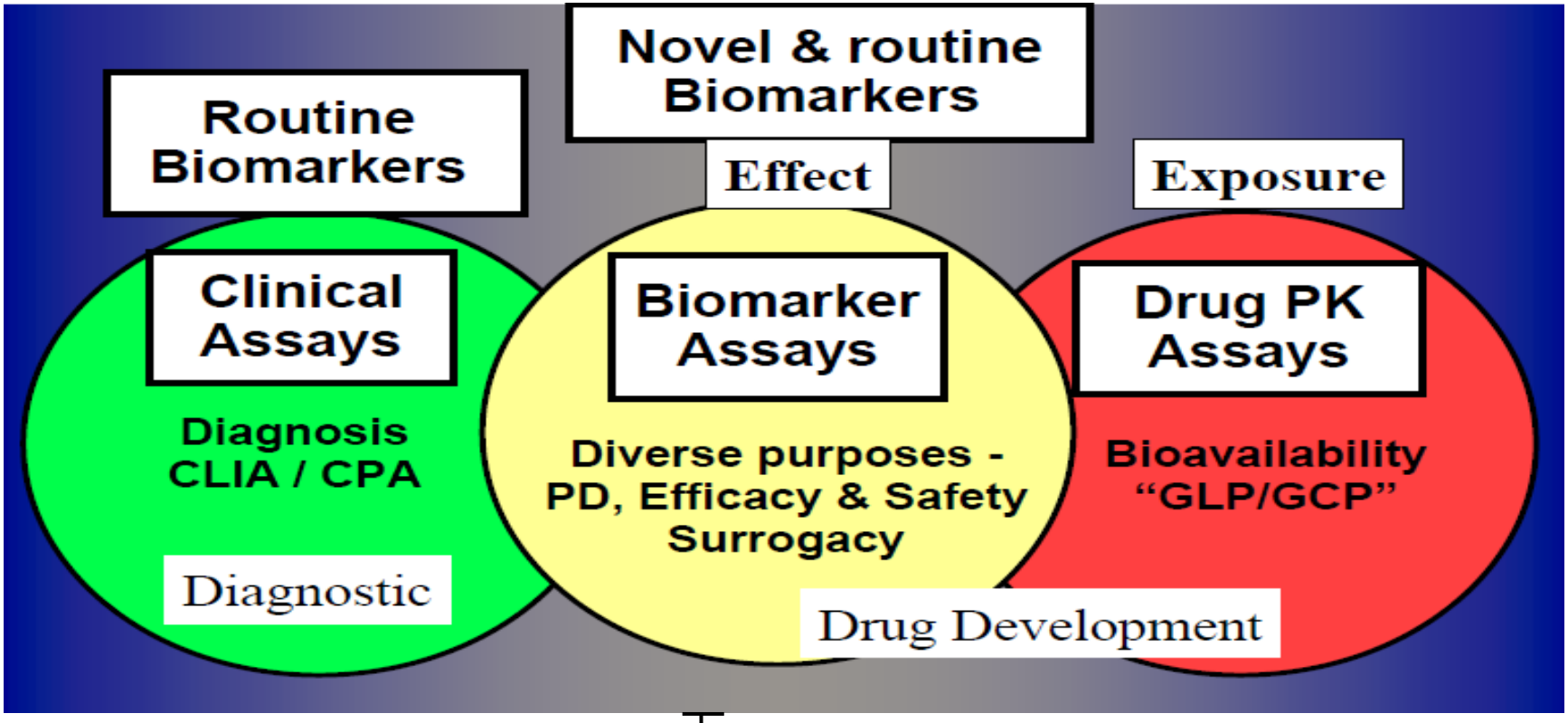
REVIEW: Fit for Purpose Biomarker Validation
What is the purpose for each assay?



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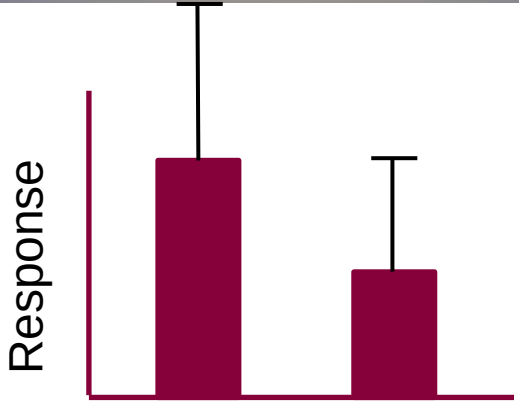
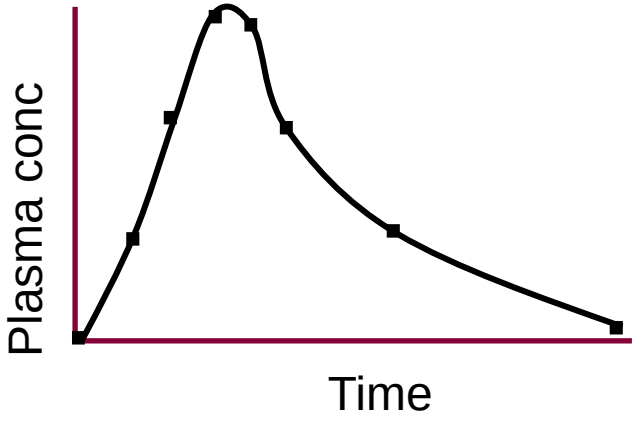
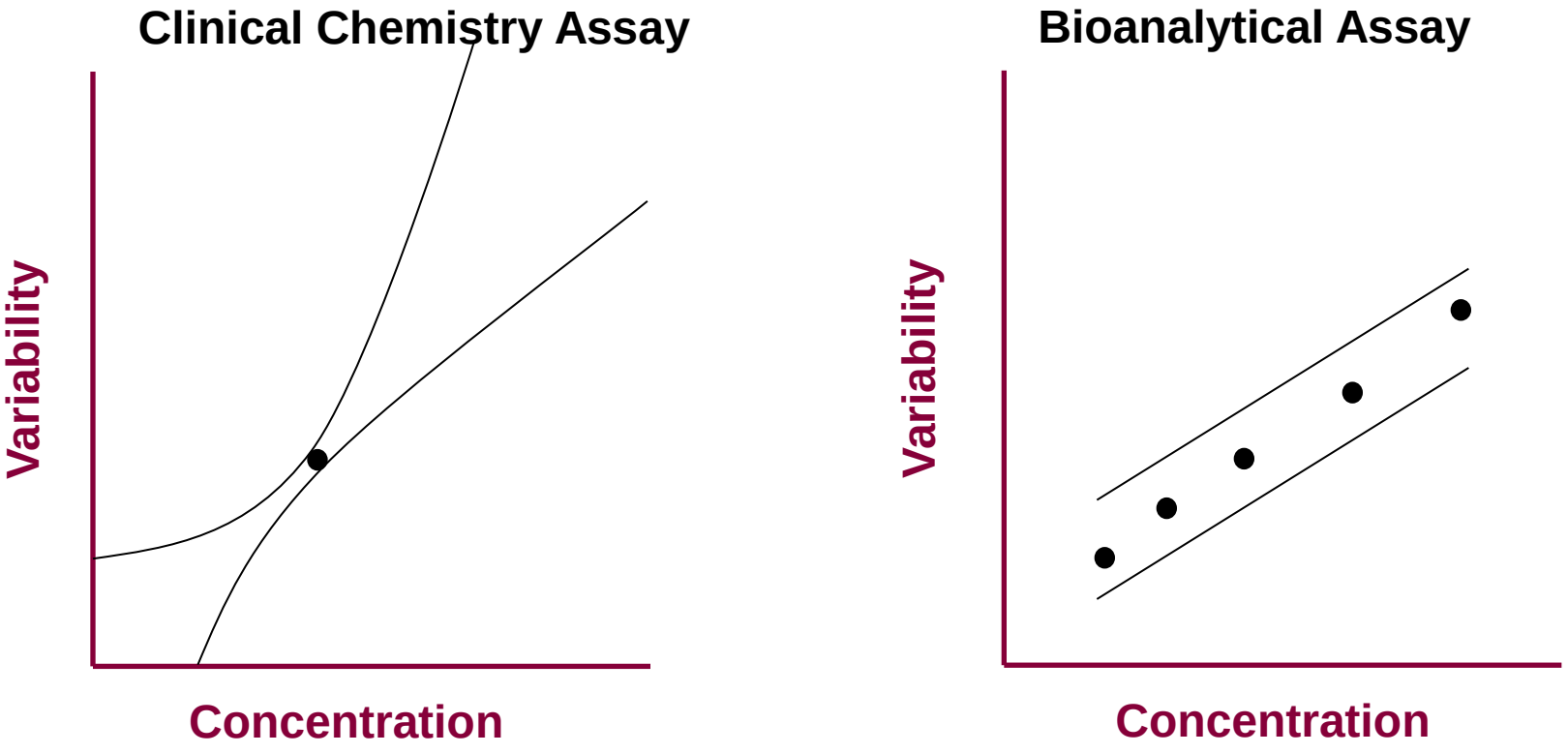




Precision - Assay variability comparison



The design of bioanalytical assays using multiple standards produces constant variability over a large concentration range.

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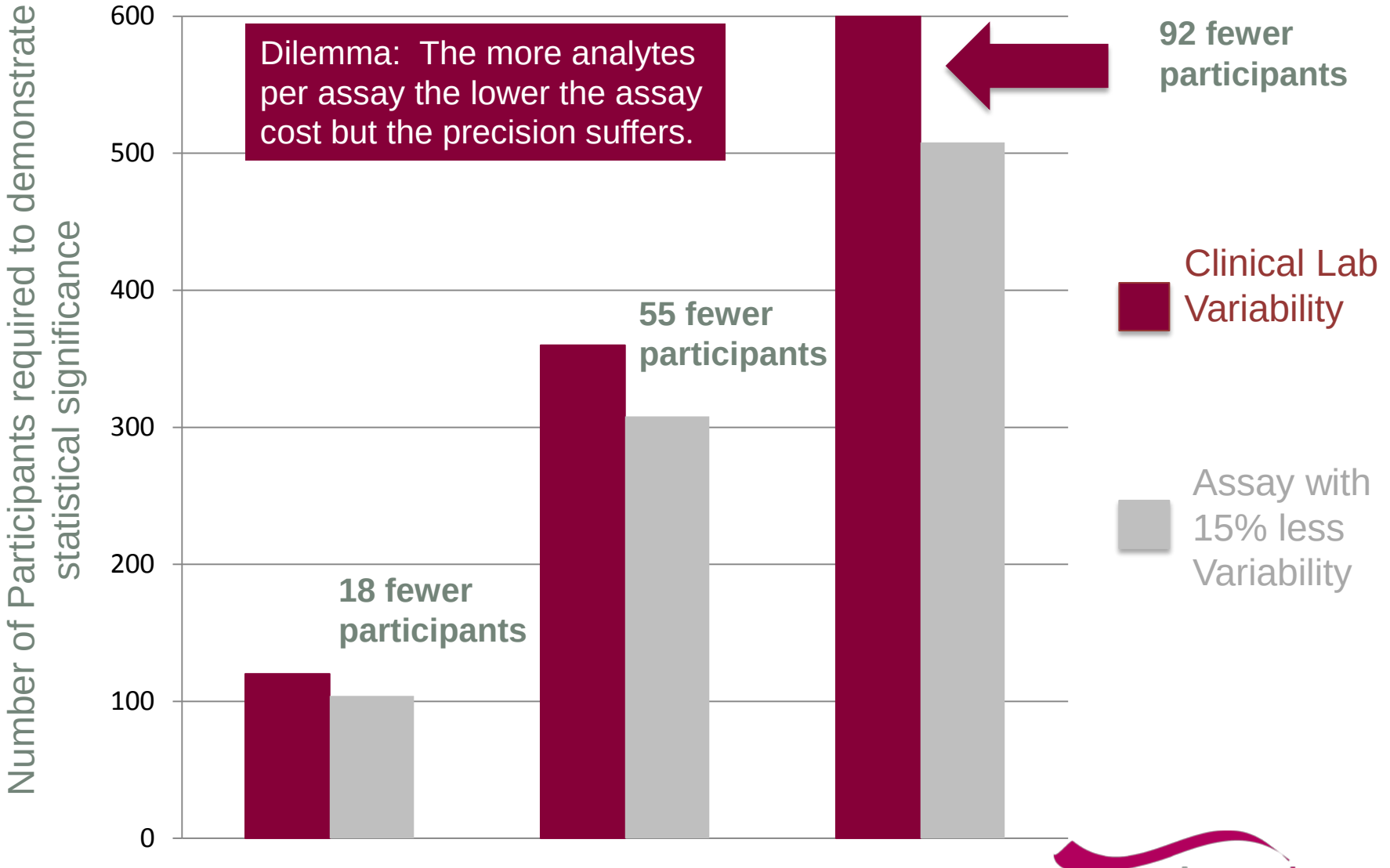
Method Validation – Precision
COST SAVINGS: Fewer Participants



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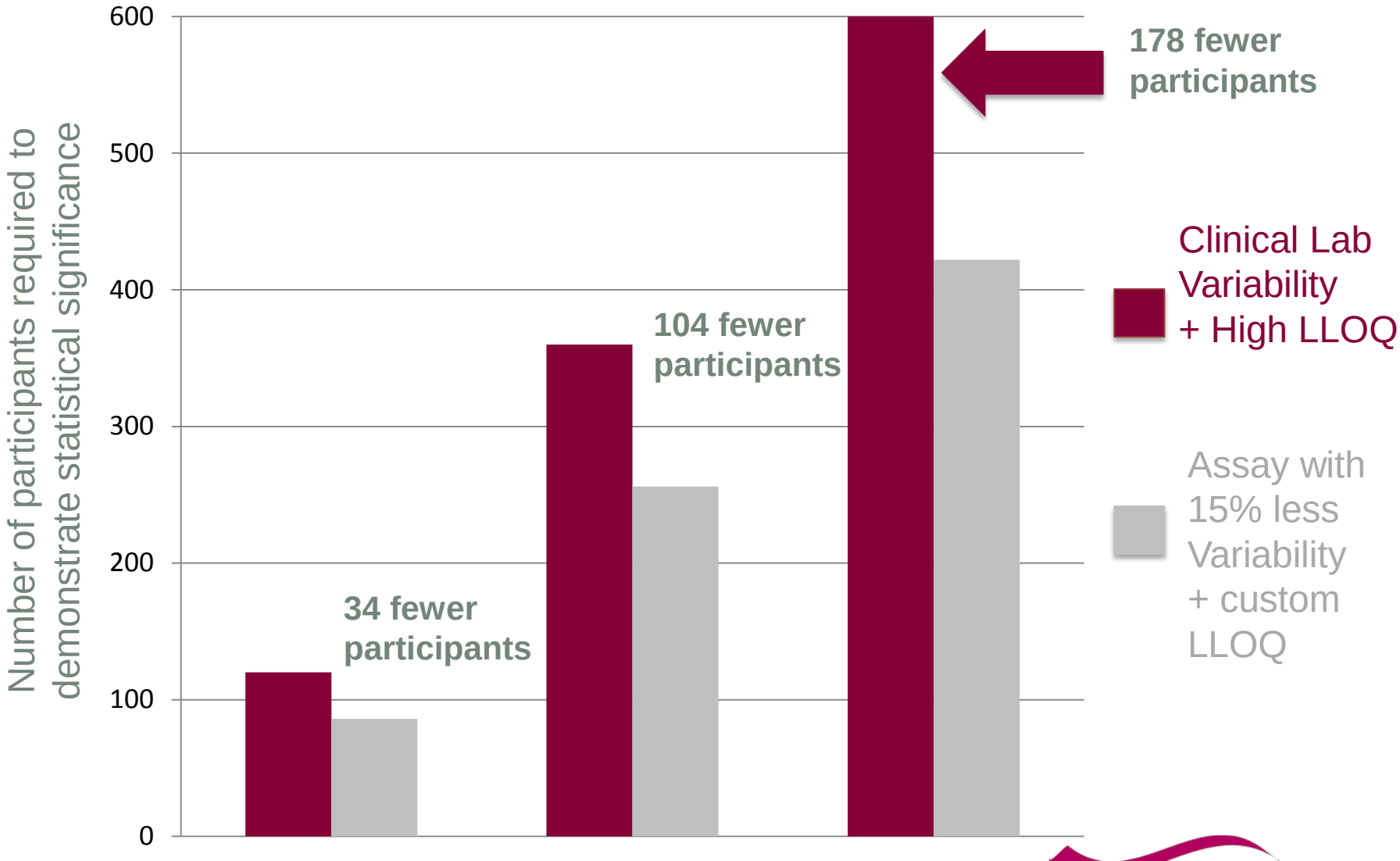
Method Validation: Sensitivity + Precision
COST SAVINGS: Almost additive



Biomarker Evolution

Biomarker Discovery

- Testing 6 -12 biomarkers / assay
- Clinical Chemistry or Luminex for multiplexing
- High variability
- Sensitivity has not been determined
- Selectivity is not validated

Validated Biomarker

- Testing 2 -5 biomarkers / assay
- OK variability
- OK sensitivity
- Selectivity is confirmed
- Meets regulatory guidelines

Biomarker optimized for clinical program

- Testing 1- 4 biomarkers / assay
- Low variability
- Sensitivity (linear range) has been optimized for MRTP
- Customized for program / product

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Conclusion

- Do you have validated biomarker for this assay?
  - Multiple answers to that question
  - Not all validated biomarker assays are equal
- The performance of biomarker assays for statistical comparisons for MRTP development can have a direct impact upon the number of subjects required for the clinical program.

References

- “Biomarkers in Acute Lung Injury – Marking Forward Progress” Nicolas Barnett, et al. Crit Care Clin **2011**
- “Isoprostane Measurement in Plasma and Urine by Liquid Chromatography – Mass Spectrometry with One-Step Sample Preparation” Debajit Sircar, et al. Clinical Chemistry **2007**
- “F2-Isopsotanes as Novel Biomarkers for Type 2 Diabetes: a Review” Subremanian Kaviarasan et al. J Clin Biochem Nutr. **2009**

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