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Applied Translational Medicine

Fit-for-Purpose Biomarker Validation - Considerations for MRTP Applications

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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 overall cost of the study
    - Sample Collection
    - Assay sensitivity
    - Assay precision
- Conclusion: Do you have a validated biomarker that will reduce clinical costs?

Challenges to Establishing Reduced Harm

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

Premarket Tobacco Applications - FDA Guidance

- **New tobacco products (910 application)**
  - **Exposure and health risk assessments**; use patterns; initiation and cessation evaluations; addictiveness and abuse potential

- **Modified risk tobacco products (MRTP application)**
  - Risk modification order (911(g)(1) application) Demonstrates and marketed as **reduced harm or the risk** of tobacco-related disease associated with commercially marketed tobacco products
  - **Exposure modification order (911(g)(2) application) Demonstrates reduced exposure to harmful tobacco constituents** compared to currently marketed tobacco products

Fit-for-Purpose Biomarker Validation

"Validating bioanalytical methods includes performing all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix (e.g., blood, plasma, serum, or urine) is reliable and reproducible for the intended use."

- Accuracy
- Precision
- Selectivity
- Sensitivity
- Reproducibility
- Stability

- FDA 2013 Bioanalytical Method Validation Draft Guidance

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Fit-for-Purpose Biomarker Validation

Less validation may be sufficient for exploratory methods and internal decision-making.

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 2013 Bioanalytical Method Validation Draft Guidance

Biomarker Evolution

Time 

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 your purpose
- Customized for program / product

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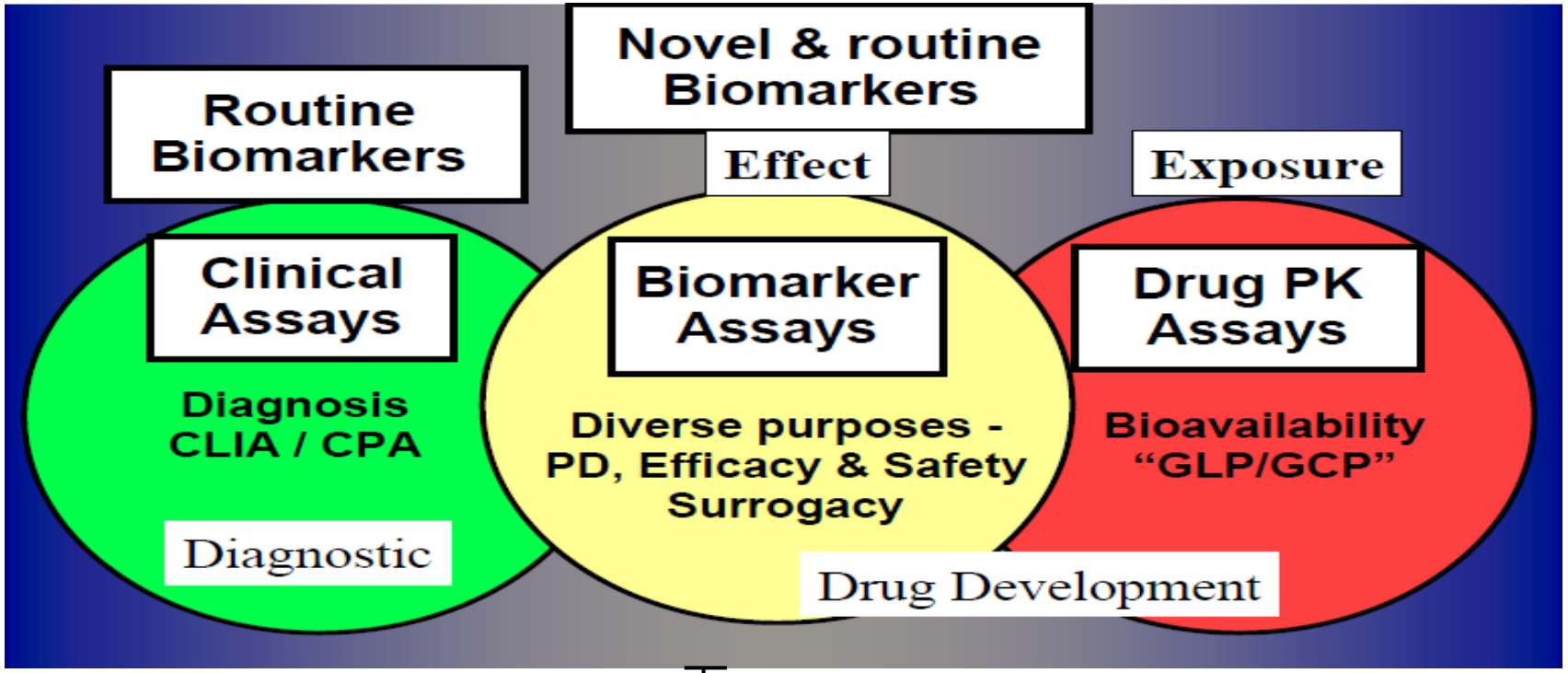
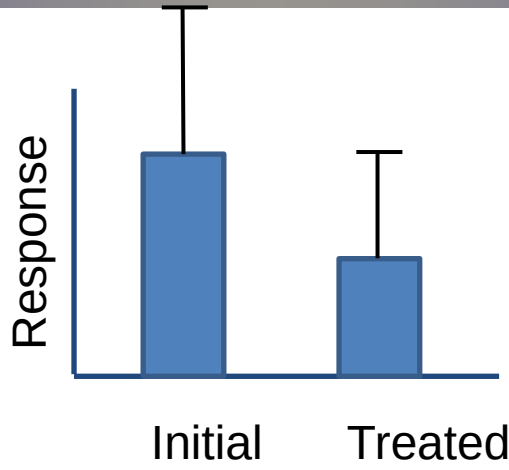
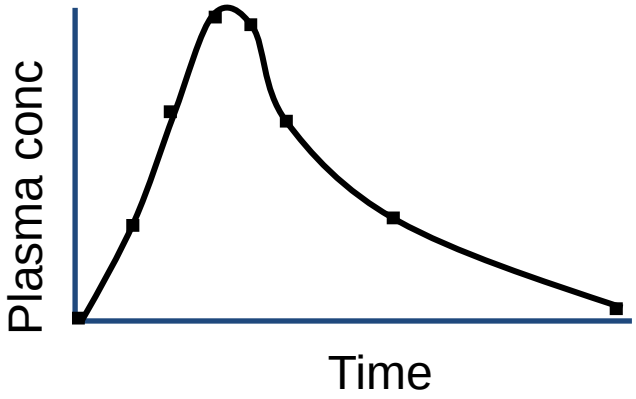
Fit for Purpose Biomarker Validation
What is the purpose for each assay?



Is the value normal or abnormal?





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

- Will this biomarker be present in the matrix?
- Is this biomarker present in the environment?
- Is this an endogenous biomarker?
- Do disease states affect the concentration of this biomarker?
- What is the intra-subject and inter-subject variability?
- Is reference material available for this biomarker?
- How much are you are willing to spend for this assay?

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

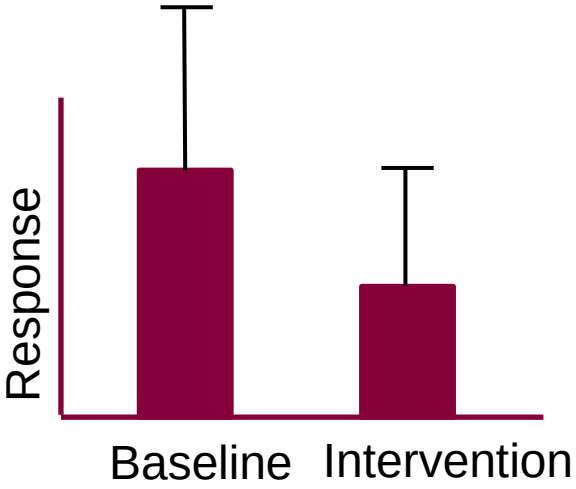
- Minimize environmental contamination:
  - o-toluidine, an aromatic amine, was shown to leach from certain urine collection containers (4X > LLOQ) and other types of plastic ware.
  - Nicotine is ubiquitous (tube contamination 20X > LLOQ) - Methanol rinsed tubes, pipettes, etc. eliminates contamination.

Method Validation – Sample Collection
COST SAVINGS

- Proper collection and sample handling requires the preparation of a detailed sample handling manual.
- If you don’t collect the samples properly to stabilize them and to eliminate contamination then don’t bother to run the study.

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

- Old LLOQ
  - 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 it should not be included in the statistics

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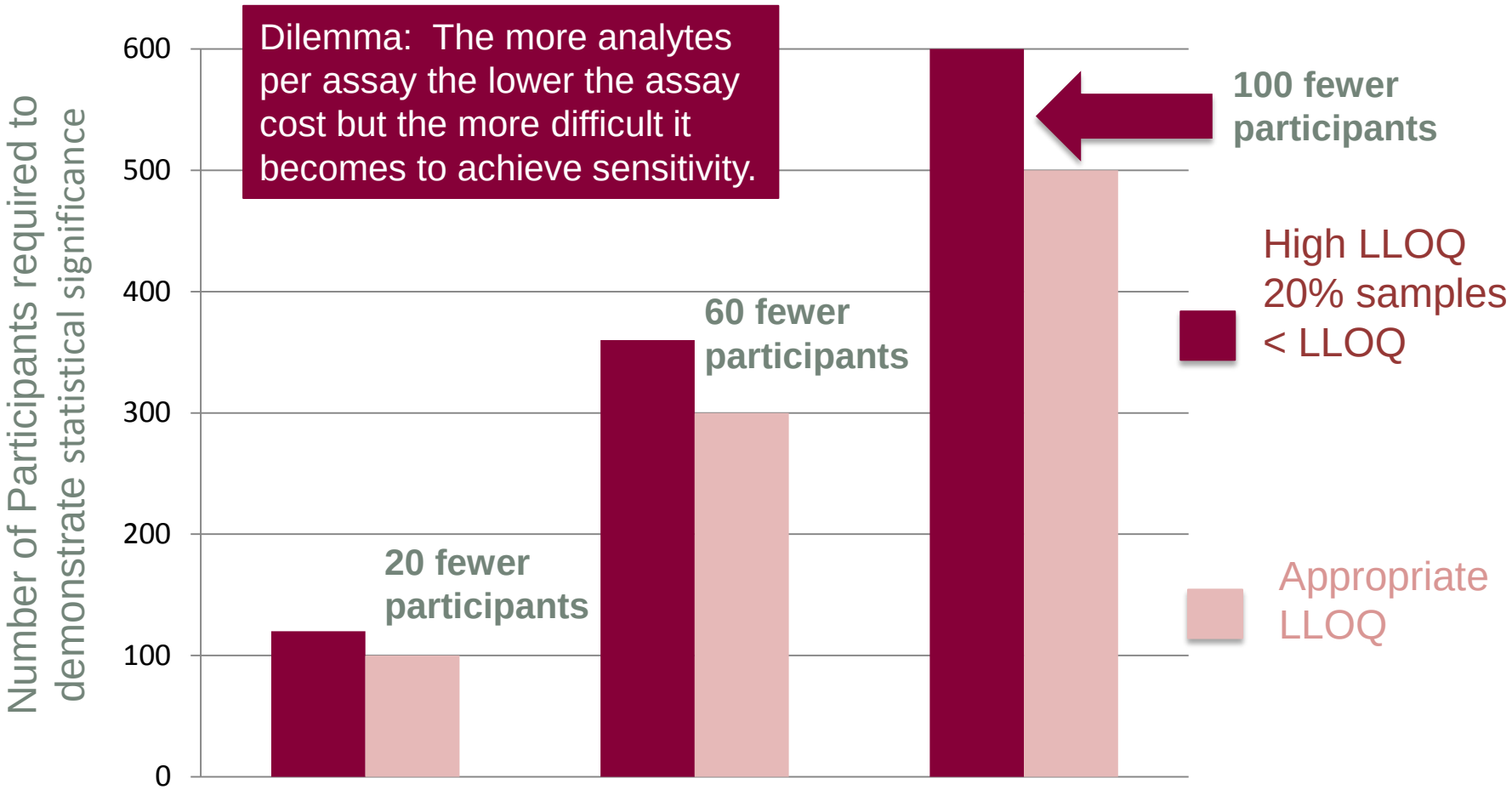
Method Validation – Sensitivity
COST SAVINGS: Fewer Participants



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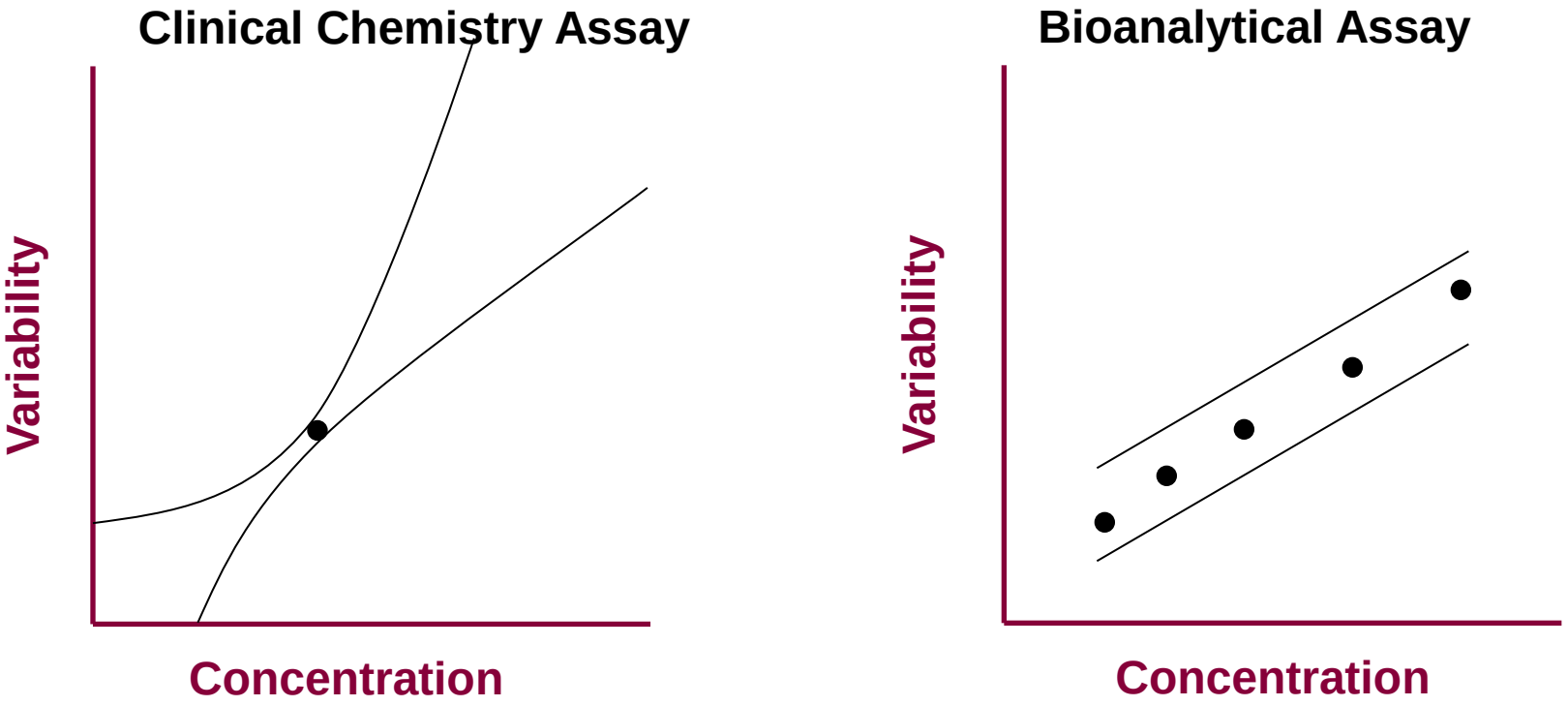
Precision - Assay variability comparison



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

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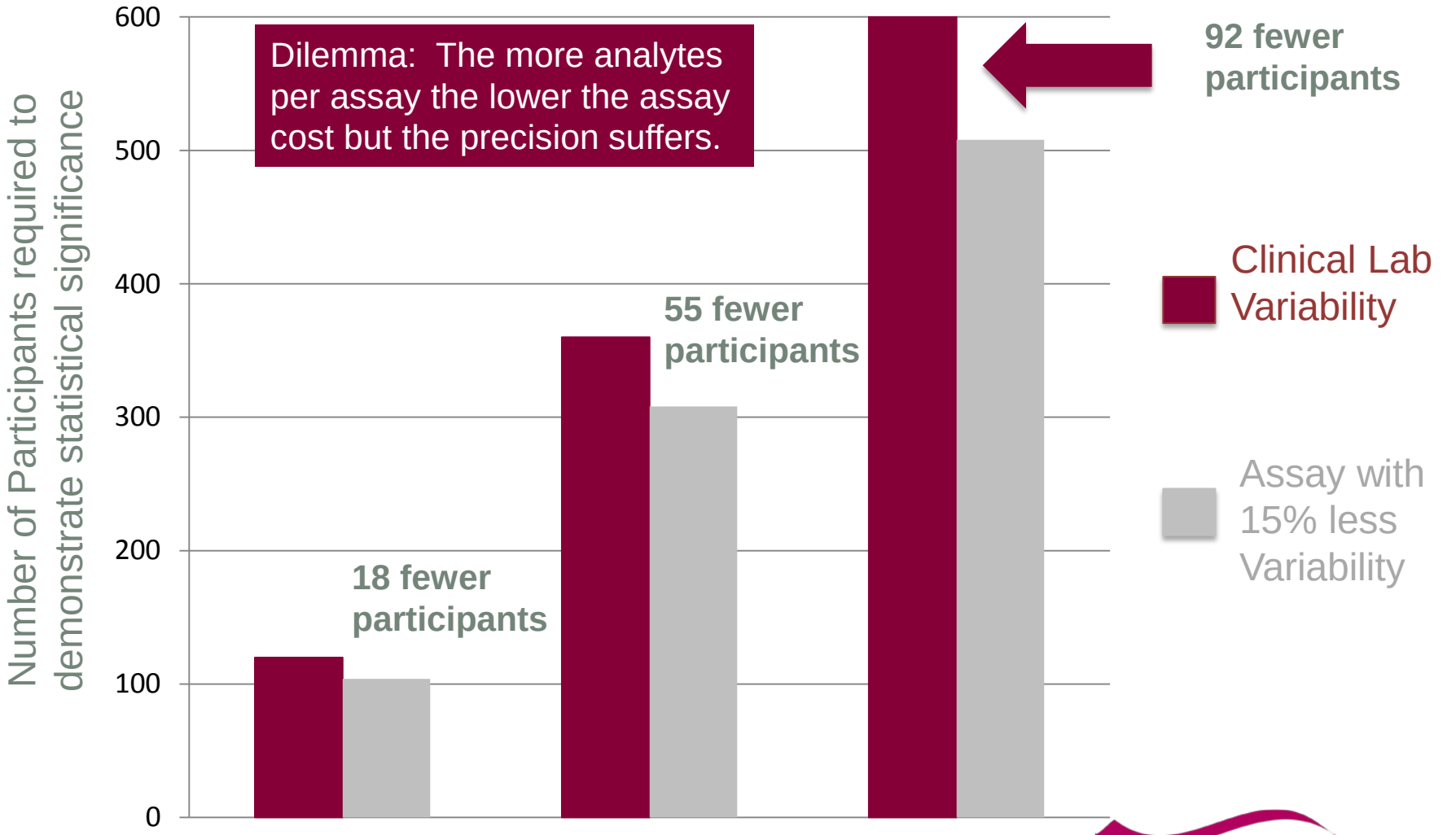
Method Validation – Precision
COST SAVINGS: Fewer Participants



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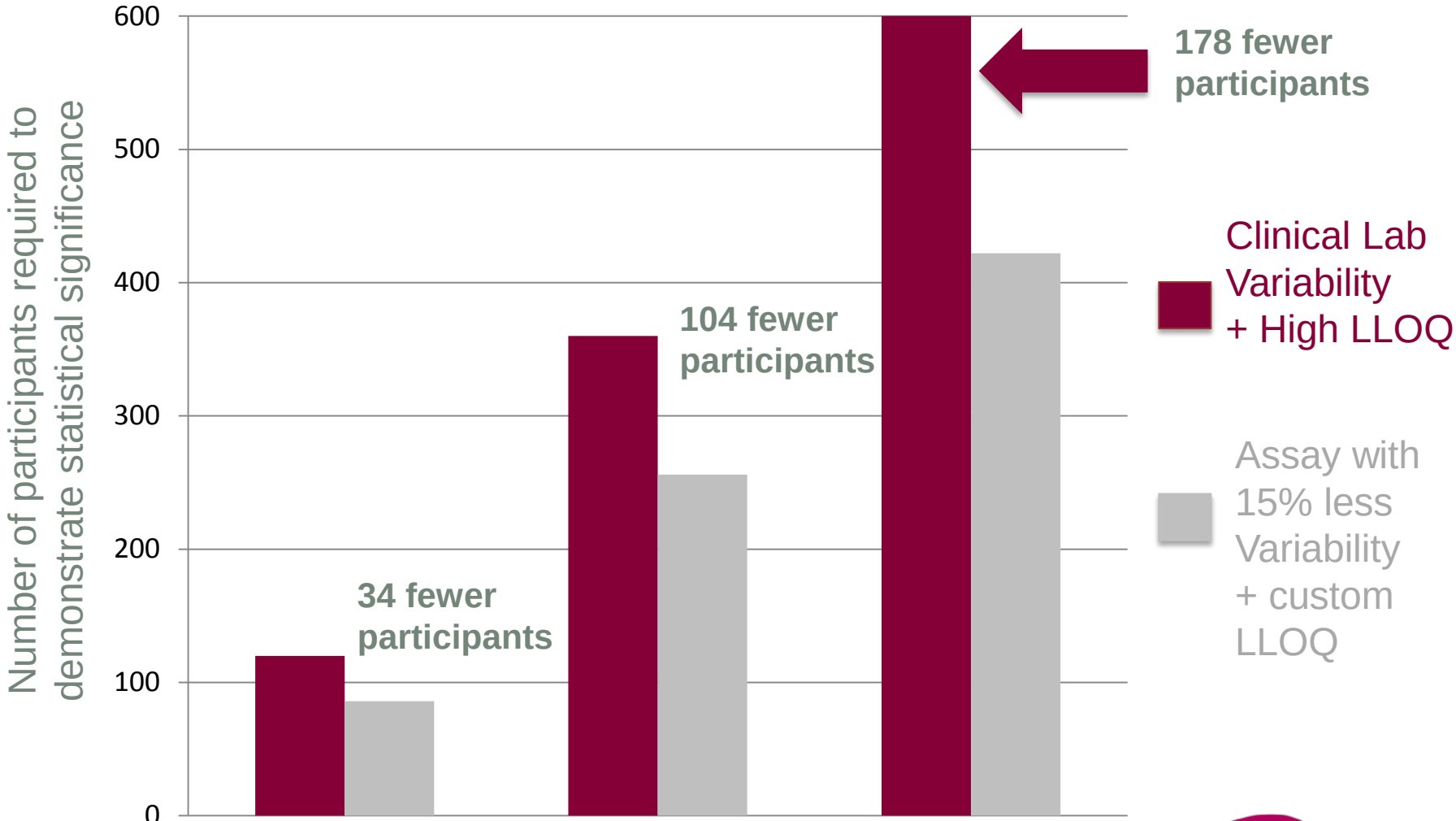
Method Validation: Sensitivity + Precision
COST SAVINGS: Almost additive



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Conclusion

- Do you have validated biomarker and does it serve your purpose?
  - 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 costs of your clinical program.