



Fit-For-Purpose Method Validation For Successful Biomarker Measurement To Support MRTP Applications

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Presentation Outline

- Definition of Modified Risk Tobacco Product (MRTP)
- Challenges to establishing MRTPs
- Biomarker as a tool to support Modified Risk Tobacco Product Application (MRTPA)
- Key considerations during biomarker method validation
- Key considerations during sample analysis
- Summary

Modified Risk Tobacco Product (MRTP)

- MRTP means “any tobacco product that is sold or distributed for use to reduce harm or the risk of tobacco-related disease associated with commercially marketed tobacco products.”
- MRTPs can be introduced under “risk modification order” or “exposure modification order”

Source: FDA Draft Guidance: Modified Risk Tobacco Product Applications

Modified Risk Tobacco Product (MRTTP)

- Risk modification requires
 - Evidence that MRTTP significantly reduces harm and the risk of tobacco related diseases to individual tobacco users
 - Benefit the health of the population as a whole
- Exposure modification requires
 - Scientific evidence is not available and cannot be made available without conducting long term epidemiological studies for an application under 911(g)(1)
 - Scientific evidence of substantial overall reductions in exposure to the harmful substance(s)
 - Benefit the health of the population as a whole

Challenges to Establishing Reduced Harm

- The concept of harm reduction/modified risk products is complex and sensitive
- Reducing the content of the toxin \neq reduce exposure of toxin \neq reduced harm
- Harm reduction strategies must be measured by their effects on health outcome
- Most important health outcome associated with tobacco are delayed in onset
- Lack of understanding of biological events from chronic smoking to disease manifestation

Challenges to Establishing Reduced Harm

- Epidemiological studies are the “gold standard”
- Epidemiological studies require;
 - Long times due to long latency period of smoking related diseases such as cardiovascular diseases, COPD, lung cancer etc. to observe clinical end point
 - Large number of subjects with well-matched confounding factors such as age, sex, ethnicity, and other lifestyle factors
 - Subject compliance to the study protocol over long period of time
 - Low subject attrition rate

Challenges to Establishing Reduced Harm

- MRTP development requires faster and more controllable method to assess
- 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

Biomarker Definition

- ‘Biomarker’ is a good example of a term whose dictionary definition is not keeping pace with the word’s changing significance in the real world
- The US National Institutes of Health definition:
 - “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention”

Types of Biomarkers

- Biomarker of exposure - potential to interact with a biologic macromolecule
- Biomarker of biologically effective dose - binds to or alter a macromolecule
- Biomarker of biological event with potential to lead to harm - measurement of an effect attributable to exposure
- Biomarker of harm - generally diagnostic biomarkers used to identify the presence of disease

Biomarkers to Support MRTPA

- Types of biomarkers based on the intended purpose
 - Decisive
 - Supportive
 - Explorative

Biomarker validation processes are continuous and iterative, and driven by the intended purpose of the biomarker data

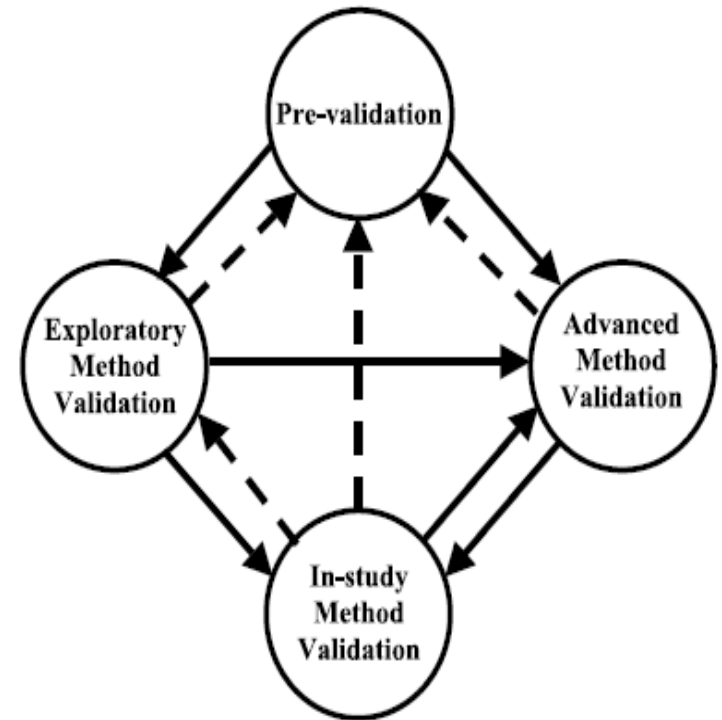


Fig: Conceptual diagram of fit-for-purpose method validation.

Source: Lee et. al (2006)

Method Validation

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

Sample Collection Procedure

- Sample collection vials must be tested for presence of analyte
 - Example- Some sample collection vials may contain 1-4 ng of nicotine
- Non-specific binding of analytes to the collection vials should be tested and detected early and resolved prior to sample collection
- Proper additives need to be chosen and adsorption test should be performed
- Allocate enough time for bioanalytical scientists to troubleshoot if non-specific binding or leaching is observed

Method Validation - Selectivity

- The analytical method should be able to differentiate the analyte of interest and IS from endogenous components in the matrix and other components in the sample
- Metabolite analysis – primarily a concern with nicotine and TSNA. As pharmacokinetic evaluations are observed with nicotine in serum or plasma, it is expected that the assay validation data demonstrate sufficient selectivity in the presence of high concentration metabolites
- In the presence of low concentration target analytes, high concentration metabolite concentrations should be supplemented to ensure conversion during collection, sample extraction, and detection does not occur
- Alternatively, back-conversion of metabolites can be checked by applying incurred sample reanalysis (ISR) or incurred sample stability (ISS)

Method Validation - Selectivity

- Additional requirements regarding medications allowed during studies has been noted. Stability testing in the presence of con-meds should be evaluated. While this is clearly not possible for all tobacco constituents, it is common practice to include selectivity testing of an OTC cocktail when evaluating assay selectivity
- Evaluation of the impact of sample hemolysis and lipemia is now expected for assays using serum or plasma samples
- Acceptance Criteria: The response in individual matrices should be no less than 20% of the LLOQ for the analyte in at least 80% of the tested individual matrices

Method Validation - Carry-over and Contamination

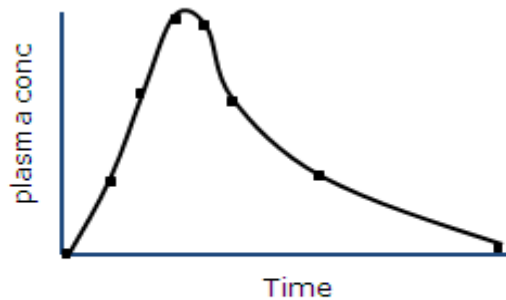
- All sources of carry-over and contamination should be minimized
 - Carry-over should be assessed by injecting blank samples after high concentration samples or calibration standard at the upper limit of quantification.
 - All possible sources of contamination should be investigated.
 - Nicotine is the most abundant organic compound emitted during smoking, deposits on indoor surfaces and lasts up to months.
 - Example: For high sensitivity nicotine method it was necessary to rinse every surface (transfer tubes, pipette tips, injection vials, etc.) with methanol to minimize contamination.
- Acceptance Criteria: The response in the blank sample should be equal or less than 20% of the LLOQ for the analyte

Method Validation - Sensitivity

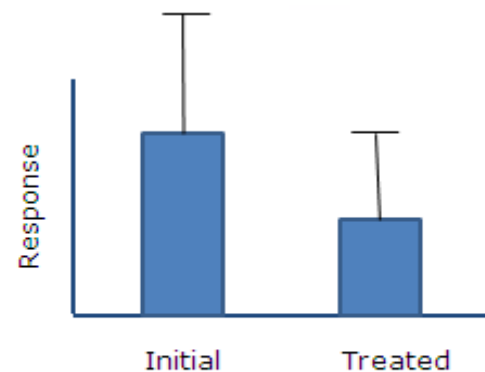
- Sensitivity (Lower Limit of Quantitation - LLOQ):
 - LLOQ is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision
 - The analyte signal of the LLOQ sample should be at least 5 times the signal of the blank sample
- For decisive (in some cases for supportive) biomarker, it is critical to have appropriate LLOQ. Just because it might be difficult or expensive, it does not provide a waiver
 - Celerion has one of the most sensitive assays for the measurement of NNN
 - It was found that >20% of the clinical samples were below LLOQ. It is difficult (if not impossible) to do comparative statistics when a lot of the results are <LLOQ
 - New method is in development using state-of –the-art MS technology

Method Validation - Precision

- The precision of the analytical method describes the closeness of the repeated individual measures of analyte
- Acceptance criteria: The coefficient of variations (CV) for with-in run imprecision and between-run imprecision should not exceed 15% (20% at LLOQ)
- For decisive biomarkers it may not be sufficient!
- Improving assay precision for biomarkers can have a significant impact on statistical results

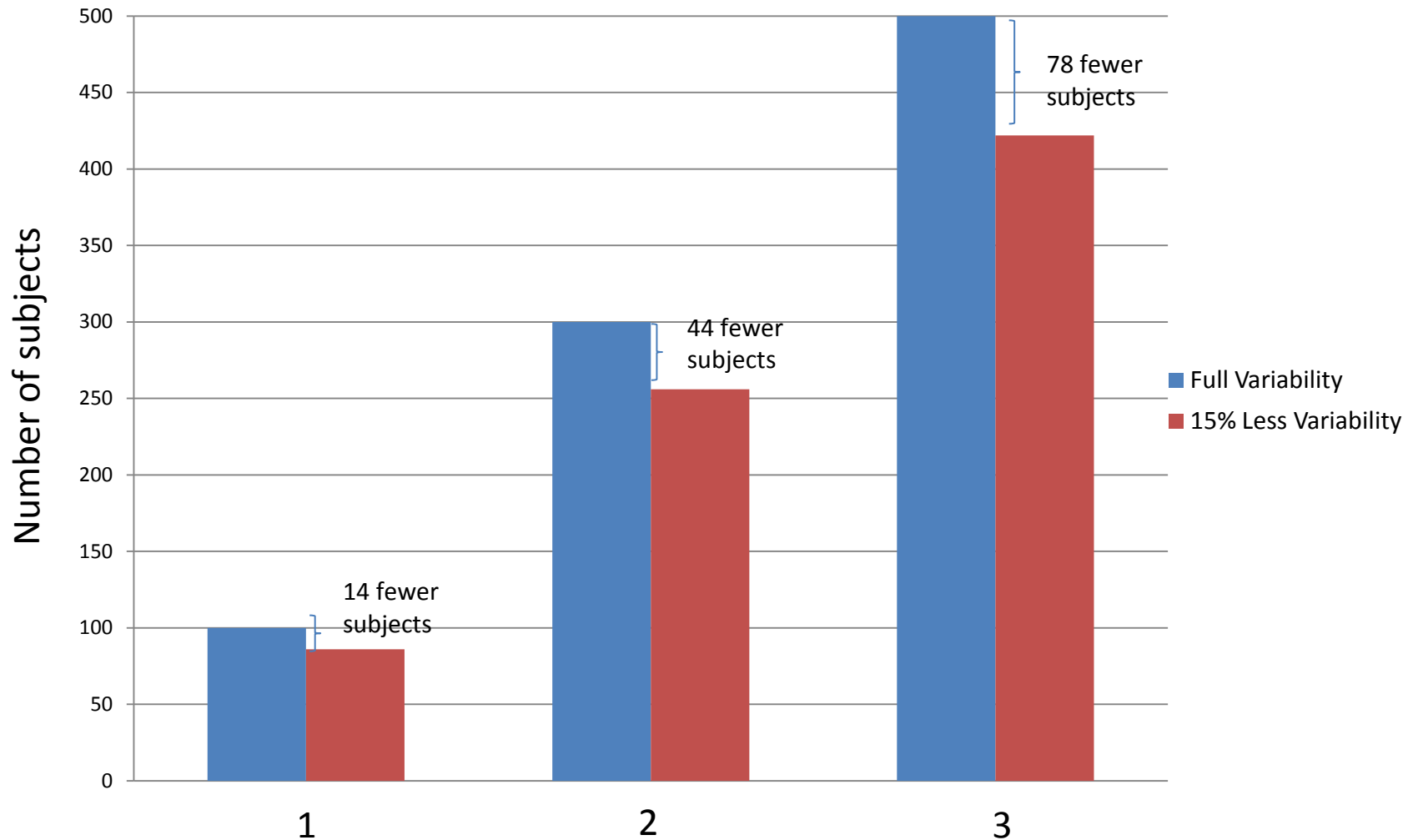


For PK needs $\pm 15\%$ precision is OK



For stats $\pm 15\%$ precision may not be OK

Assay Variability's Impact on Number of Subjects



Method Validation and Cross-Validation

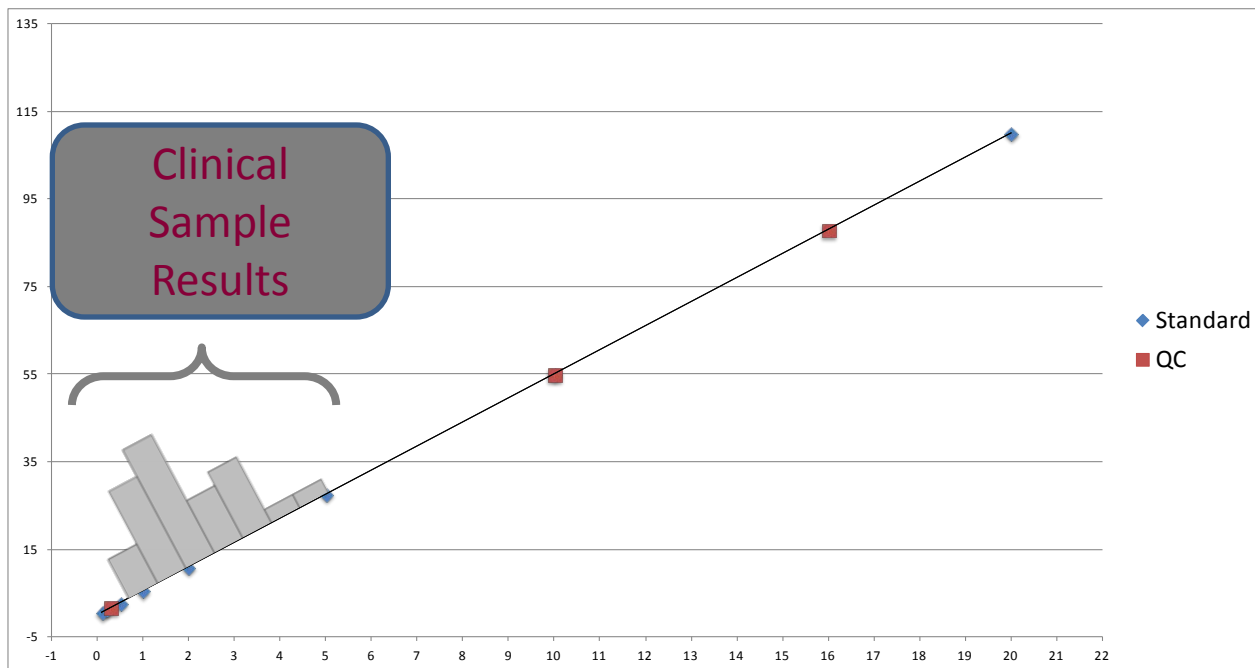
Minimum Requirements

- At Celerion the following tests are required per SOP for Bioanalytical Method Validation

Validation Test	Initial Validation	Cross-validation
Precision and Accuracy	X	X
Multi-lot Matrix Effect / Matrix Factor	X	
Blank Matrix Selectivity	X	X
Hemolyzed Sample Matrix Effect	X	
Turbid Sample Matrix Effect	X	
Sensitivity Assessment	X	X
Recovery Assessment	X	
Long-term stability	X	
Freeze/thaw and Short-term stability	X	X
Post Preparative Stability	X	
Stock Stability	X	
Sample Collection and Handling Stability	X	
Frozen Aliquot Storage Stability	X	
Dilution Integrity	X	X
Processed Sample Integrity	X	
Stress Test	X	X
Sample Shipping Stability	X	

Sample Analysis – Standards & QCs

- Each batch must contain its own standard calibration curve
 - Minimum of six different calibrator concentrations
 - Range of the standard curve should reflect the expected range of the study sample concentrations

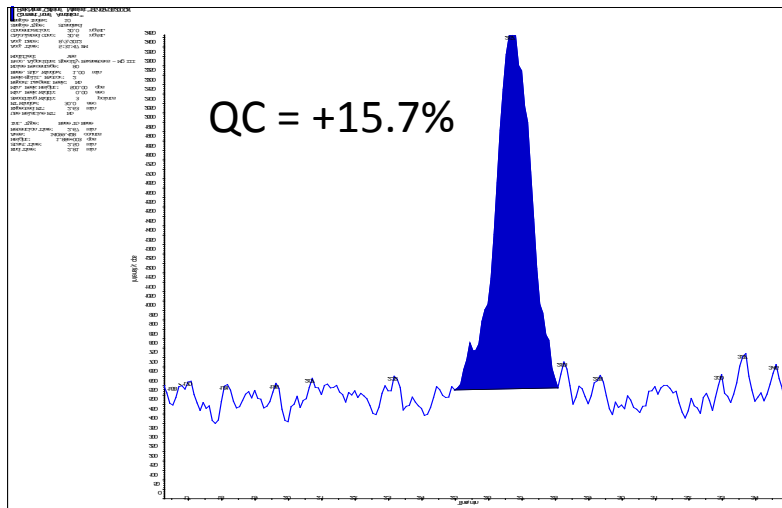


Sample Analysis – Standards & QCs

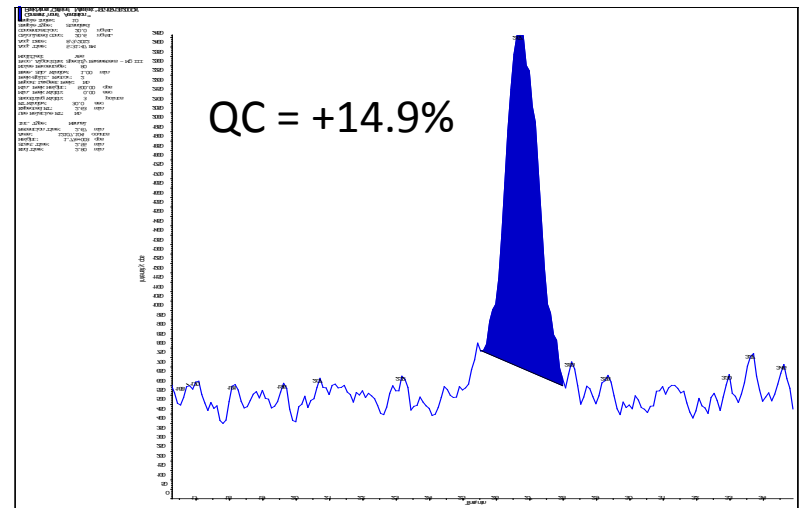
- Each batch must contain its own standard calibration curve
 - Minimum of six different calibrator concentrations
 - Range of the standard curve should reflect the expected range of the study sample concentrations
- Each batch must contain a minimum of 3 QC concentrations in duplicate (low, middle and high)
- What's the difference between standards and QCs?
 - Philosophically – standards create the calibration curve and QCs demonstrate that non-standards can be accurately measured – so they must be different
 - Different weighings – getting standards and QCs to match each other
 - QCs are stored with study samples to verify sample integrity

Sample Analysis: Chromatographic Integration

- Big Deal – the vast majority of serious audit findings involve scientists modifying integration parameters for QCs to get them to pass acceptance criteria



Consistent with other samples
Original computer generated



Inconsistent with other samples
Manually drawn

Sample Analysis – Chromatographic Integration

- Big Deal – A significant number of serious audit findings involve scientists modifying integration parameters for QCs to get them to pass acceptance criteria.
- Best Practice – Review all chromatographic integration and manually redraw baselines then “lock-it-down” prior to performing regression analysis. It should be very difficult to modify integration following regression analysis.

Sample Analysis - Acceptance Criteria

- Standard Curve Acceptance
 - Must use the same regression model and weighting option that was chosen during method validation
 - Must have an SOP in place that describes how to reject standard(s)
 - Only sample concentrations that are within the range of your accepted standards can be reported
- QC Acceptance
 - The predicted concentration of 2/3 of the QC samples must be within 15% of their nominal concentration (20% for ligand binding assays)
 - At least 50% of the QCs must be accepted at each QC level

Sample Analysis: Batch Acceptance Criteria

- Blank (no internal standard) and standard zero samples
 - Prepared with matrix that was free of interferences
 - At least 50% of these samples must have a response that is <20% of the response of the lowest standard – reject low standard
- Sample Dilution
 - Can only report sample concentrations that fall within the range of the standard curve
 - Sample concentrations that exceed the concentration of the highest standard must be diluted
 - Must prepare dilution QCs and dilute them using the same respective dilution (for example, 1/10 dilution)
 - Dilution QCs are used for acceptance of the diluted samples
 - 50% of dilution QCs must be within 15% of their nominal concentration

Sample Analysis: Incurred Sample Reproducibility (ISR)

- Purpose is to demonstrate that re-assaying study samples is reproducible.
- Recommended by FDA and EMA
- Generally accepted practice:
 - minimum 20 samples
 - If $n < 1000$ samples then reanalyze 10%
 - If $n > 1000$ samples then reanalyze 100 samples + 5% of samples beyond 1000
 - Select more subjects with fewer samples per subject
 - Acceptance Criteria: The difference between the repeat value and the original value $< 20\%$ ($< 30\%$ for ligand binding assays) for 2/3 of the samples **Is this OK for biomarkers when precision is so important?**

Sample Analysis: ISR

- Investigation of failed ISR testing has found the following common root causes
 - Analyte stability
 - Failure to bridge reference standard materials/stocks/standards
 - Metabolite conversion or selectivity concerns
 - Analytical technique failure in the laboratory (i.e. dilution error or performance variability)
- While the original intent was to demonstrate consistent method performance, failure due to assay variability has infrequently been observed.

Sample Analysis – Reporting Sample Concentrations

- Demonstrate the sample analysis plan was followed
- Must have an SOP to select samples for re-analysis
- Must show that the acceptance criteria for each batch was met
- Demonstrate inter-batch reproducibility: List all of the QCs by batch and perform statistical analysis (% mean deviation and %CV) on each QC level for the study – study precision
- Report sample concentrations that were < the concentration of the lowest standard as < LLOQ
- Report sample concentrations that were > the concentration of the highest standard as >ULOQ
- Prepare a table by batch as either passed or failed

Summary

- Evidence that MRTP significantly reduces harm and the risk of tobacco related diseases to individual tobacco users and benefit the health of the population as a whole is REQUIRED.
- The profiles of number of biomarkers can be used to support reduced harm from MRTPs.
- Choosing appropriate panel of biomarkers is critical to the success of MRTP applications
- Fit-for-purpose Method Validation in a GLP-compliant lab is critical to the success of MRTP Applications
- Bioanalytical methods must be selective, accurate, precise, stable and documented!
- Principles of GLP along with most up to date industry practices should be utilized for sample processing and data reporting
- Incurred Sample Reanalysis (ISR) analysis a requirement
- **Just because it might be difficult or expensive does not provide waiver for advanced method validation**

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Questions or Comments?

