Fit-for-Purpose Biomarker Assay Validation: From Concept to Practices

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Outline of discussions

- Introduction - Concept of Fit-for-Purpose method validation of biomarker ligand binding assay
- Practices
  - Pre-analytical considerations
  - Major challenges of biomarker method validation that is different from those of protein biotheraputics.
  - Method feasibility and optimization
  - Exploratory vs. Advanced method validation
  - Illustrations of critical analytical issues

Acknowledgement

- American Association of Pharmaceutical Scientists (AAPS) Ligand Binding Assay Bioanalytical Focus Group.
- Colleagues at Amgen PKDM
- Ron Bowsher

Motivation & Purposes of Biomarker Assay Validation

- Biomarkers can play an important role in evaluating the safety and/or effectiveness of a new medical product
- It is critical to ensure the integrity of data from these assays
- A fit-for-purpose approach should be used for the wide varieties of purposes during drug development
- Full Validation required to support a regulatory action (e.g., pivotal safety or effectiveness, labeled dosing instructions)
- Validation of appropriate extent for early drug development (e.g., candidate selection, go-no-go decisions, proof-of-concept)
- The majority of biomarkers are endogenous proteins, which are generally analyzed by ligand binding assays (LBA)

FDA Draft Guidance for Industry Bioanalytical Method Validation 2013
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

Fit-for-Purpose Concept

‘Fit-for-Purpose’ Biomarker Assay Validation

Pharmaceutical Research, Volume 23, No. 2, February 2006 (© 2006)
DOI: 10.1007/s11095-005-9045-3

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

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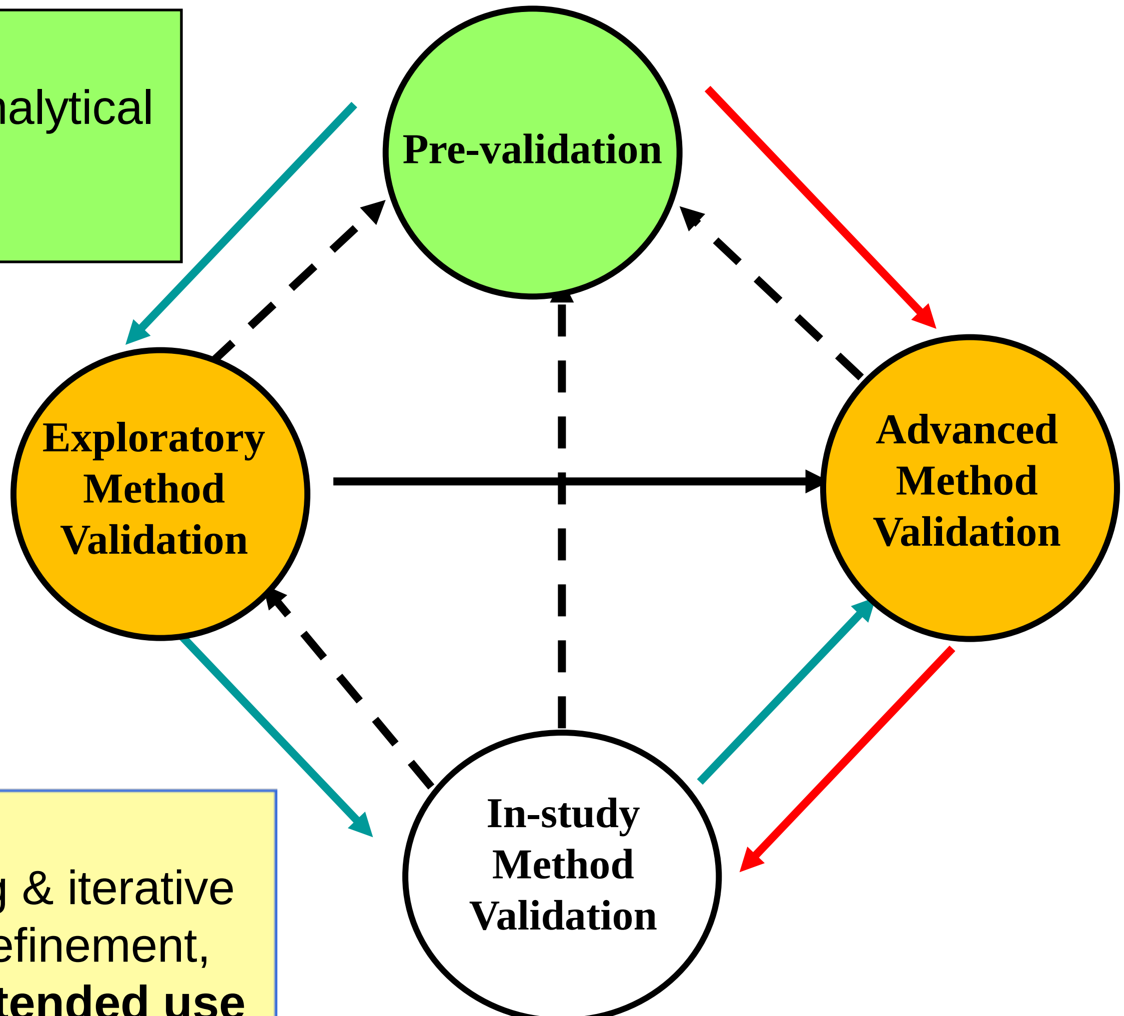
Abstract. Despite major advances in modern drug discovery and development, the number of new drug approvals has not kept pace with the increased cost of their development. Increasingly, innovative uses of biomarkers are employed in an attempt to speed new drugs to market. Still, widespread adoption of biomarkers is impeded by limited experience interpreting biomarker data and an unclear regulatory climate. Key differences preclude the direct application of existing validation paradigms for drug analysis to biomarker research. Following the AAPS 2003 Biomarker Workshop (J. W. Lee, R. S. Weiner, J. M. Sailstad, *et al.* Method validation and measurement of biomarkers in nonclinical and clinical samples in drug development. A conference report. *Pharm Res* **22**:499–511, 2005), these and other critical issues were addressed. A practical, iterative, “fit-for-purpose” approach to biomarker method development and validation is proposed, keeping in mind the intended use of the data and the attendant regulatory requirements associated with that use. Sample analysis within this context of fit-for-purpose method development and validation are well suited for successful biomarker implementation, allowing increased use of biomarkers in drug development.

Lee *et al*., *Pharm Res* 2006; 23:312-328.

Conceptual diagram of Fit-for-Purpose method validation

<u>Pre-validation:</u>

- Pre-Analytical and Analytical Method Feasibility
- Method Optimization



<u>Method Validation:</u>

- Continuous, evolving & iterative
- Graded process of refinement, dependent on the **intended use**

Lee et al., *Pharm Res* 2006; 23:312-328.

Biomarker Analytical Validation

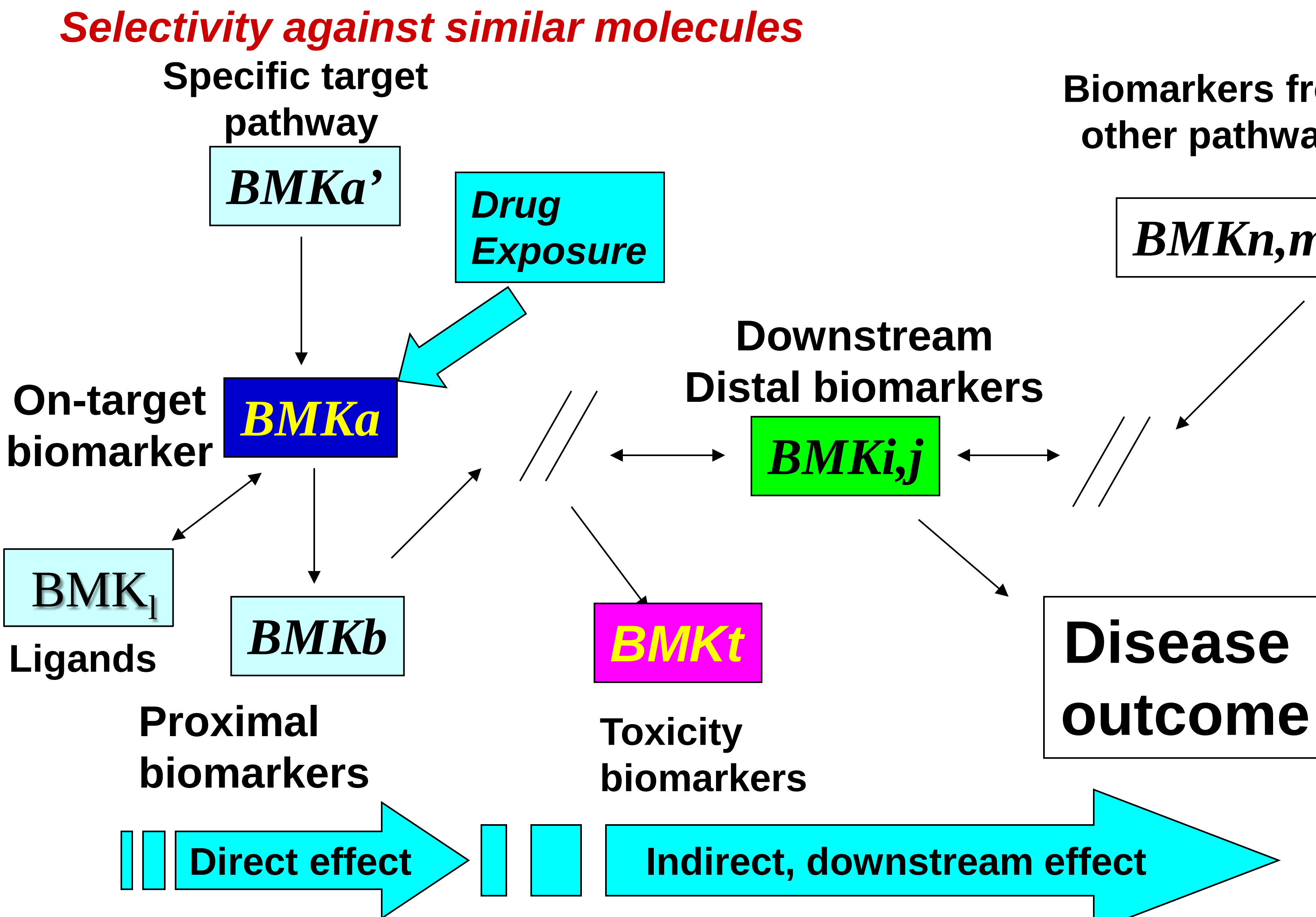
- **Exploratory** – Method validation that is less rigorous, but adequate to meet study needs
  ….*not for submission to regulatory agencies*.

- **Advanced** – Method validation of more intensity and thorough investigation both in the validation tasks and documentation.
  …for submission to regulatory agencies for drug approval.

Lee *et al*., *Pharm Res* 2006; 23:312-328.

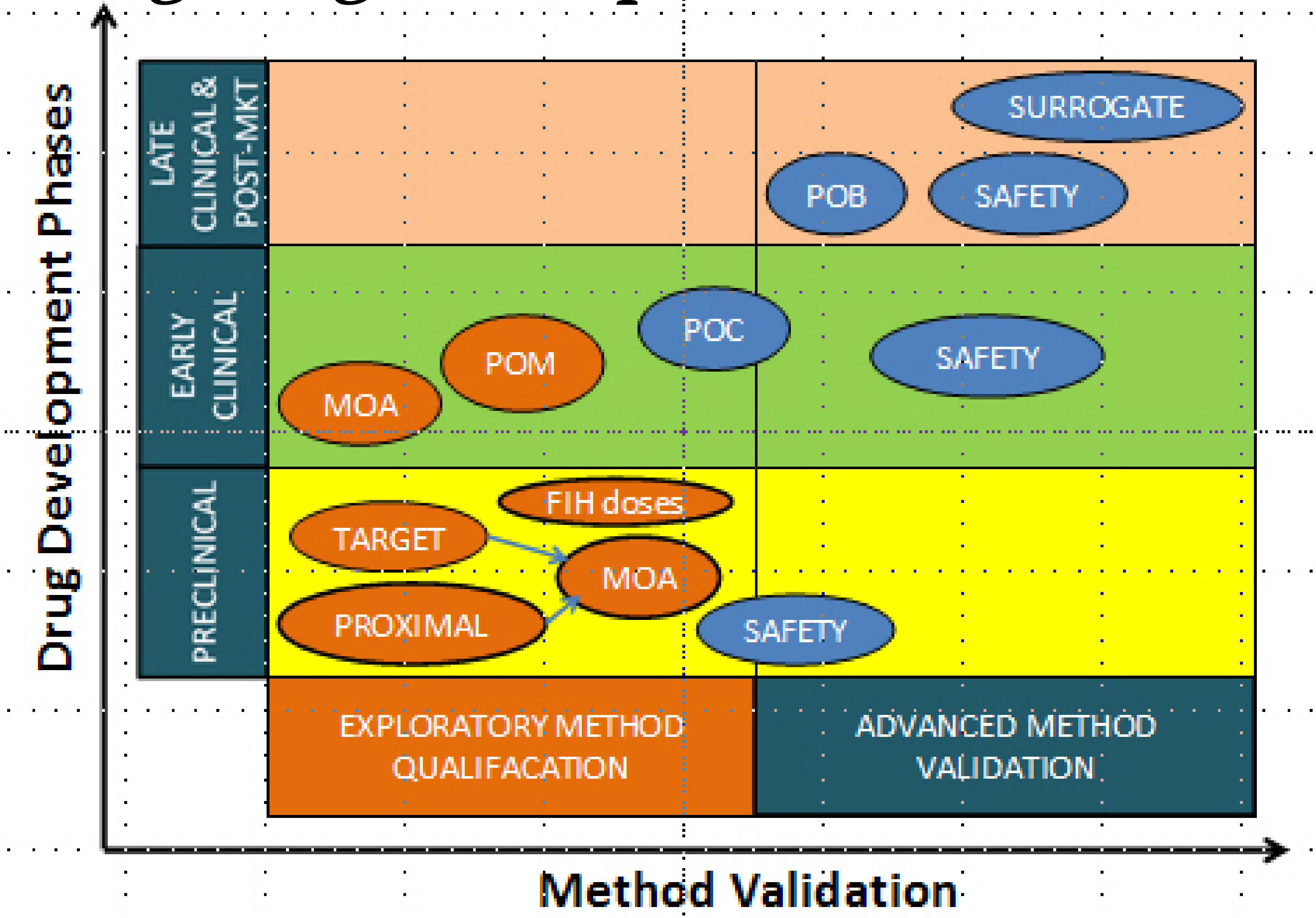
Practices

Biomarkers related to disease state and drug effects



Lag time of PD effect after dosing, single vs. multiple doses

Exploratory & Advanced method validation during drug development



Khan *et al. Bioanlysis* 7(2) 229-42, 2015

Example: Biomarkers during early clinical development of Denosumab

Biomarkers	Commercial kit or in-house	Intended Purpose	Method validation
Target biomarker a	In-house	PoM	Exploratory
Proximal biomarker b	Research kit	PoM	Exploratory
Distal biomarker c	Diagnostic kit	PoB, dose selection	Advanced
Distal biomarker d	Diagnostic kit	PoB, dose selection	Advanced
Distal biomarker e	Diagnostic kit	PoB	Advanced
Distal biomarker f	Diagnostic kit	PoB	Advanced

Schematic of decisions & processes

Work Plan
Define purpose of study & biomarker measurements
Which biomarker(s) and in what biologic matrix to be included in the study?
Exploratory or advanced application?

Pre-analytic sample integrity

Method development & validation
Reference standard & reagents
Calibrator matrix
Initial assay range & sensitivity
Quality Controls
Selectivity, parallelism
Precision & Accuracy
Matrix controls

Factors affecting biomarker dynamics and data interpretation

- Sample collection or storage conditions:
  - Convert upstream forms to the biomarker of interest, resulting in overestimation
  - Degradation of the biomarker, resulting in low levels.
- Binding of a biotherapeutic to the target biomarker requires the decision whether to measure "free" or "total" (free + bound) forms
  - "Free" levels are very low – challenge in method development
  - "Total" may provide information on compensatory feedback or drug protection effect of the biomarker
- *In vivo* conversion to downstream forms
  - Is any of these forms bioactive?
  - Options to develop "bioactive" (or epitope-specific specific immuno) assays vs. chemical (or species-specific immuno) assay of each bioactive species.

These should be considered and discussed with the project team

Basic Pre-Analytical Considerations

- Information from literature, vendor and discovery team
- Disparity of biomarker concentrations/activities in targeted disease population from normal; expected contraction from drug effect
- Biomarker concentration fluctuation with time (daylight and season), age, gender, body mass (or fat).
- Available methods and reagents
- How important will be the decision to the company?

Sources of biomarker LBA methods and reagents

Developed by Pharma companies

- Originated in discovery-research programs
- Typically for target and/or proximal biomarkers
- Method validated by the users for intended purpose

Apply Clinical Diagnostic Commercial Device or Kits

Clinical-grade Diagnostics

- FDA approved and CLIA regulated
- 510 (K) or equivalent
- Closed system, no modification

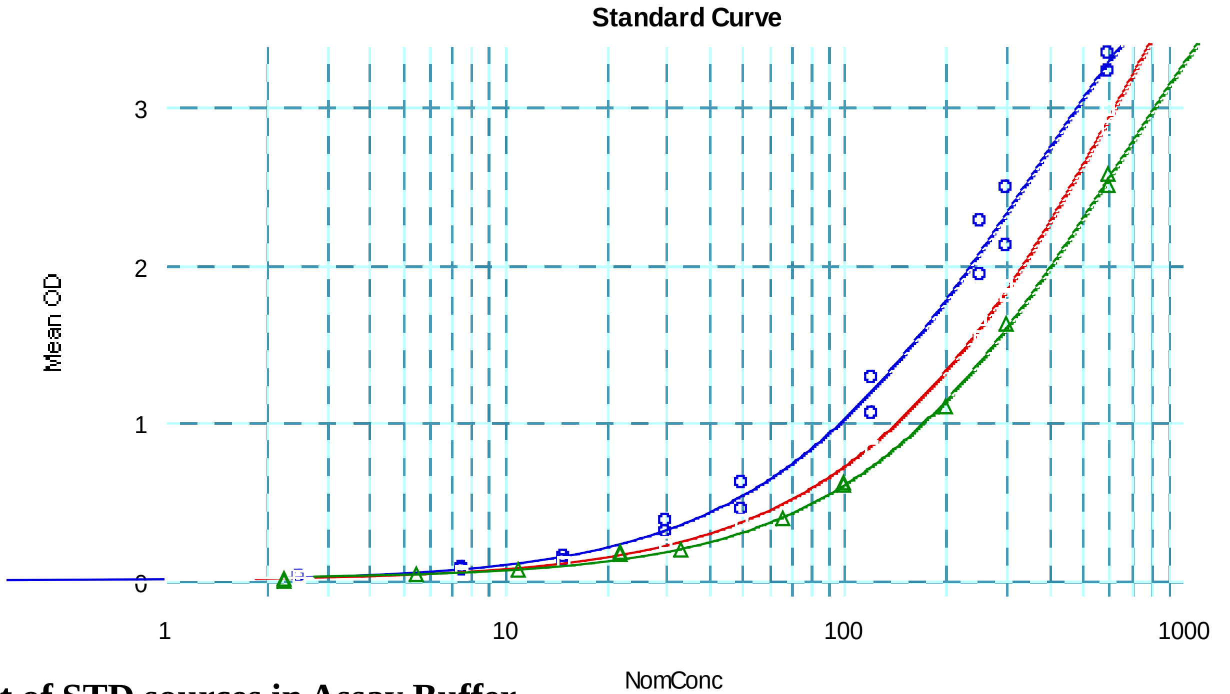
Research Use Only (RUO)

- Not FDA approved
- Manual assays
- Intended for research only
- Flexible for modifications

What are the main challenges of biomarker assay that are different from those of biotherapeutics?

- Subjected to biological variability (e.g. diurnal effect, diseases)
- Reference standard limitations – impure, not well characterized, not fully represent the endogenous biomarker
- Analyte-free biological matrix may not be found, a substituted matrix is used for calibrators
- Parallelism required to show equivalent performance of: recombinant reference ≈ endogenous; substitute matrix ≈ biological
- Matrix Controls (MC) should be made to reflect those of incurred samples
- Lot-to-lot variability of diagnostic kit performance

Kit reference standard may be inconsistent & lack documentation - Example



Test of STD sources in Assay Buffer
Green: Kit-Std   Blue: Kit-bulk Std   Red: In-house Reference std

Advantage of In-house ref standard:

- Ample supply
- Lot consistency
- Documentation: Certificate of stability and purity/structural analysis

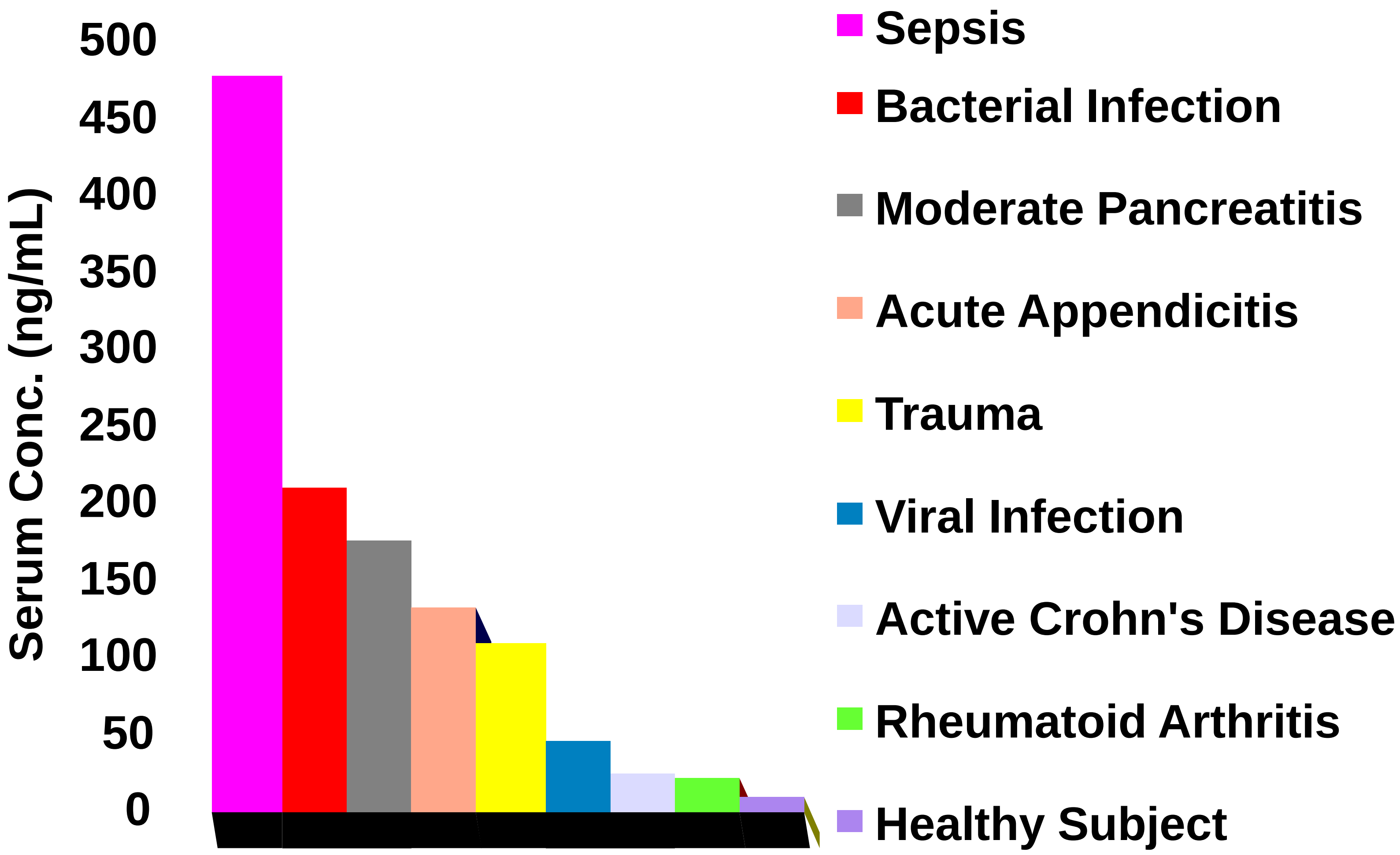
Validation samples and quality controls of biotherapeutics and biomarkers

- **Purposes**
  - Validation sample (VS) – Used during pre-study validation to characterize the method being validated, at least 4 levels including LLOQ, Low, mid and high QCs.
  - Quality control sample (QCs) – Low, mid and high QCs used during in-study validation to monitor the performance, and to accept/reject an assay run.

- **Preparation**
  - In the same biological matrix as subject samples. *This may not be possible for biomarkers with substantial endogenous amounts*.
  - Concentration range should cover the expected levels of target populations
  - Test parallelism to justify using spike samples as VS/QC

- ***Matrix Controls (MC) for biomarkers*** *– Pooled from authentic samples to reflect study samples, at least 2 levels.*

Biological biomarker variations in different diseases
Example of an inflammatory biomarker IL6



Calibrator ranges and MCs can be different for different target disease

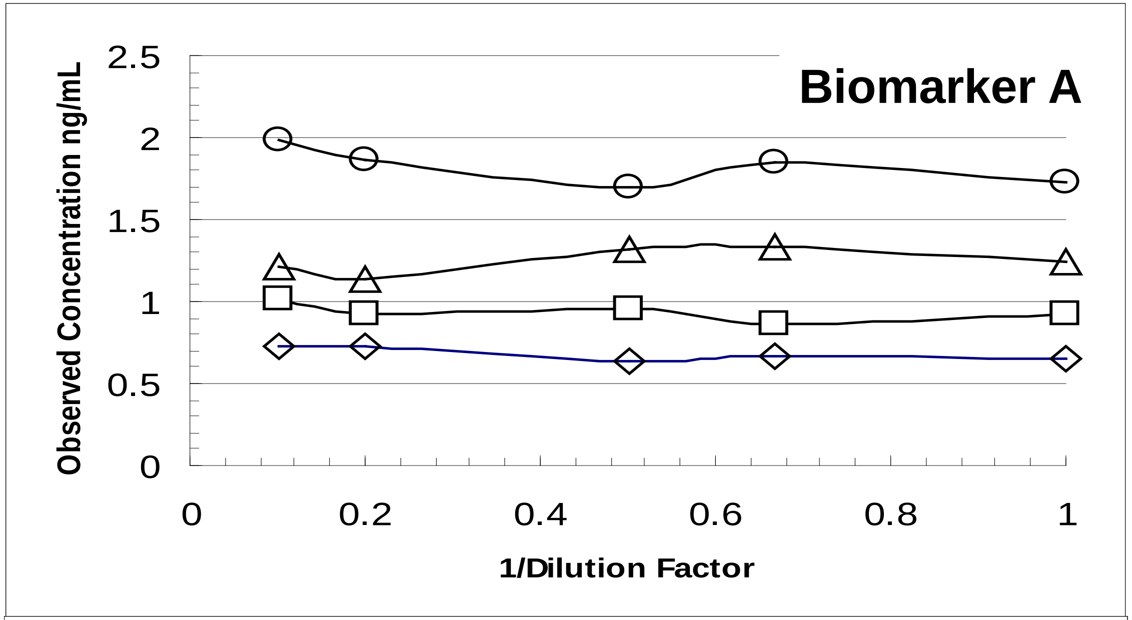
From Dr. Ron Bowsher

Exploratory Validation basic elements

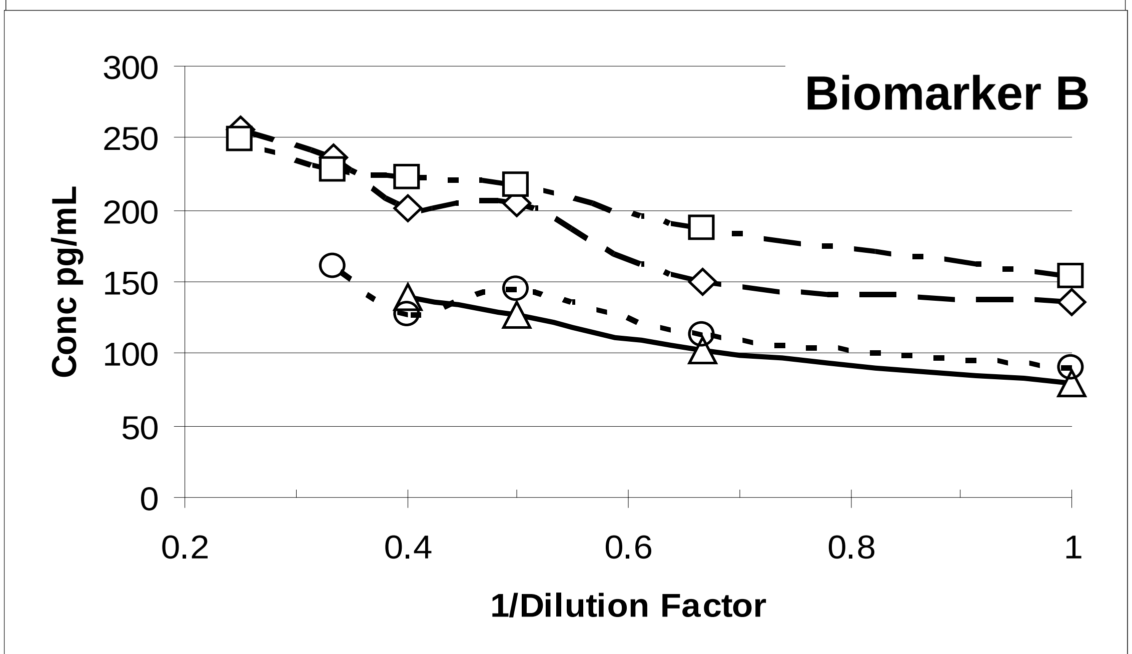
- ***What are the minimum requirements?***
- **Recommendations:**
  - Parallelism, or spike recovery
  - Validation samples reflecting clinical sample matrix and target range
  - Assay working range established with standards and validation samples
  - Sample collection integrity
  - Bench-top stability
  - Precision & Accuracy data from 3 validation runs

Example of Parallelism Tests

Multiple matrix lots
Multiple dilution factors
Dilute with standard matrix



Results acceptable



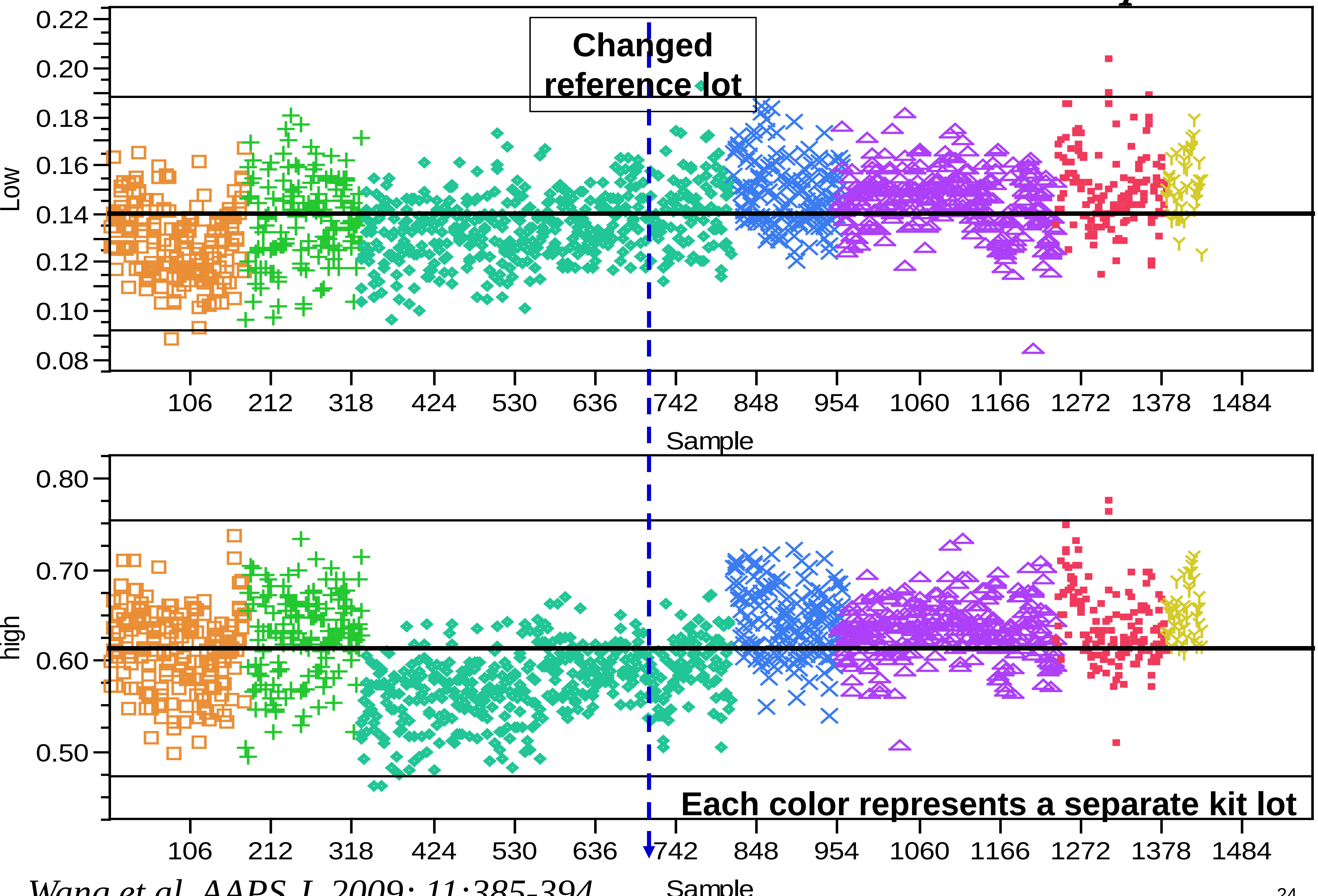
Results failed

Essential considerations for advanced validation and in-study validation

- **Advanced Method Validation**
  - Reagents and reference material stability & inventory control over time span of the program
  - Target range from incurred samples, relative accuracy/recovery from multiple donors
  - Assay performance evaluation from more validation runs (≥6)
  - Long term stability
  - Extensive testing of assay interferences (matrices, concomitants)
  - Robustness (reagent & change control)
  - Learn from in-study validation data of pilot study
  - Beware of biological variability from commercial or in-house samples.

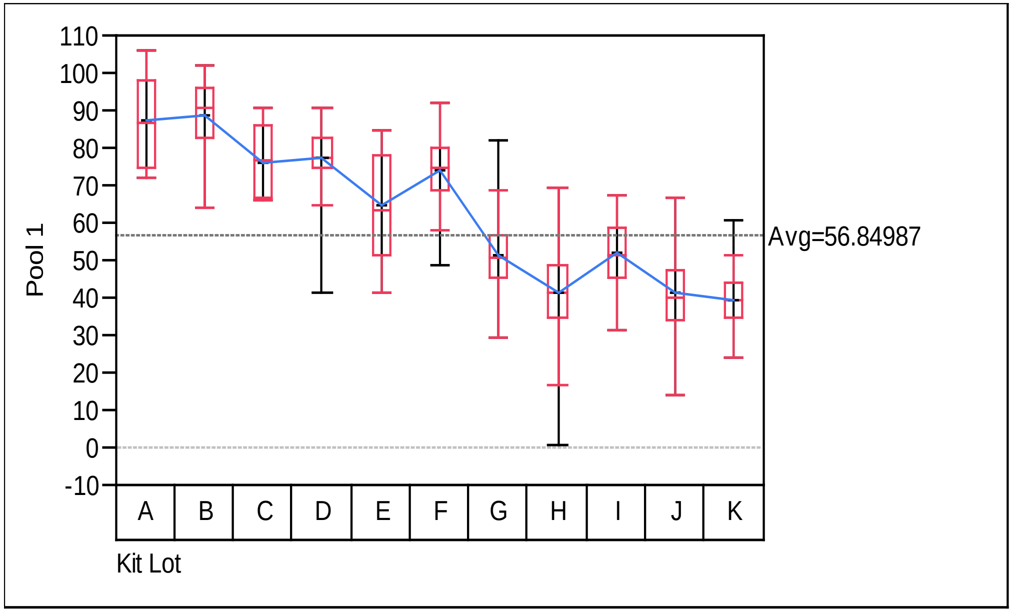
- **In-Study Method Validation**
  - Statistic tools for Acceptance criteria
  - MC from incurred samples vs. spiked samples

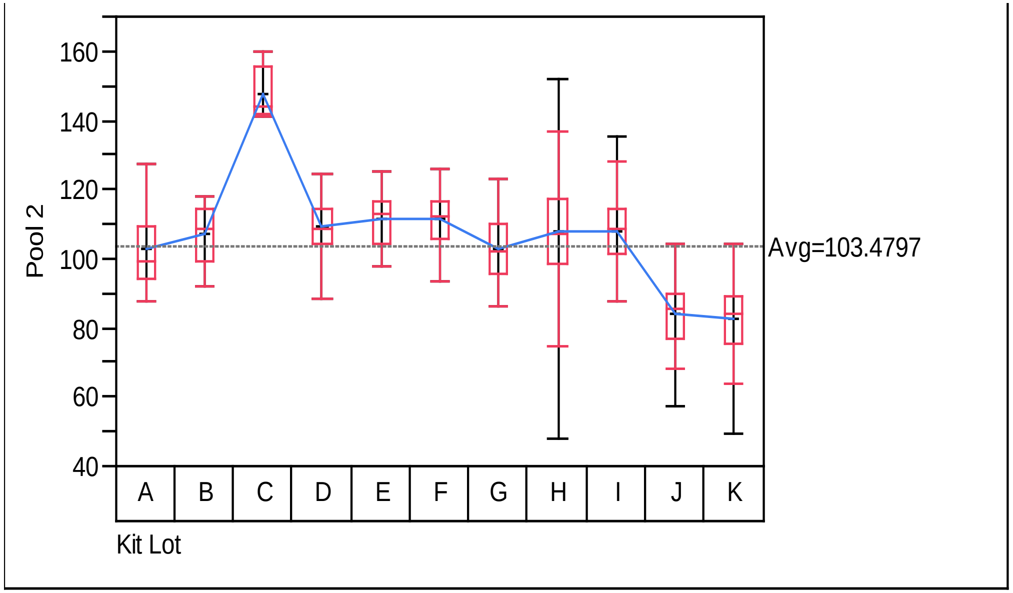
Matrix Controls in an Advanced Validation - Example



Wang et al. AAPS J. 2009; 11:385-394.

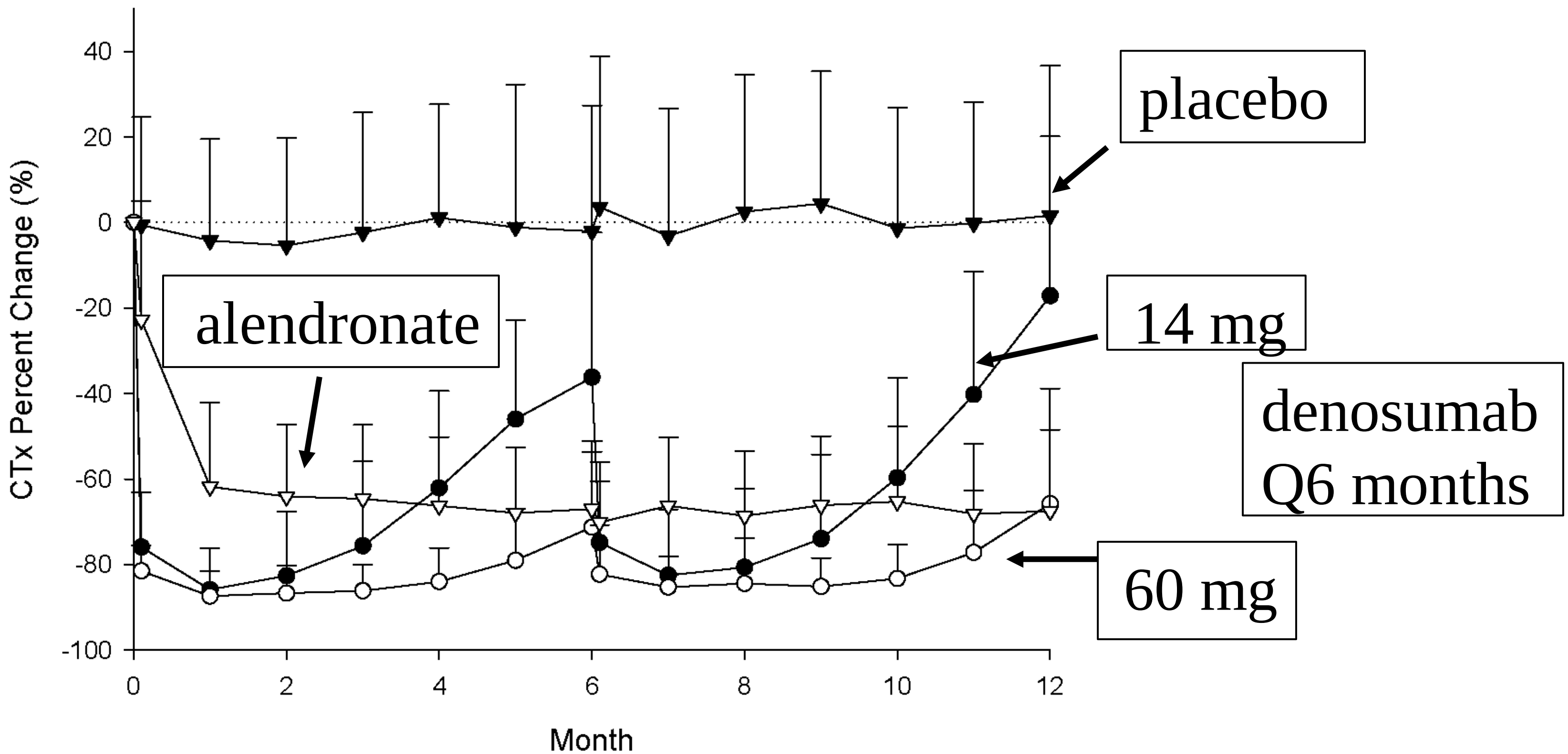
Matrix Controls in an Exploratory Validation – Example of RUO kit lot differences





		Pool 1		Pool 2	
Kit lot no.	**N**	**Mean**	**%CV**	**Mean**	**%CV**
All	**450**	**56.9**	**32.6**	**103.5**	**14.5**
A	10	87.3	15.1	102.7	11.3
B	16	88.6	11.6	107.4	7.7
C	5	76.3	13.4	147.8	5.2
D	63	77.6	9.9	109.5	6.9
E	13	64.7	22.3	111.7	7.7
F	53	73.7	12.6	111.4	6.7
G	74	51.0	18.9	103.2	8.6
H	67	41.2	26.9	107.6	14.3
I	64	51.8	16.6	107.9	10.0
J	35	41.3	25.6	84.4	12.0
K	50	39.5	18.4	82.6	12.8

Example - Pharmacodynamic profiles of CTx % Change in patients dosed with denosumab



Dose related suppression was shown by the PD biomarker

Summary highlights

- Target-oriented working plan to guide biomarker investigation
- Assays to meet the intended purpose of the study, instead of following biotherapeutic LBA guideline for PK studies.
- Exploratory Method Validation
  - Range finding to determine appropriate range and representative QC/MC levels
  - Parallelism experiments
- Advanced Method Validation
  - Proactive plans for consistent supply of critical reagents
  - Matrix Controls to monitor assay performance and stability
- Learn from initial study data to statistically assess drug effect over the basal levels.

Thank you for your attention

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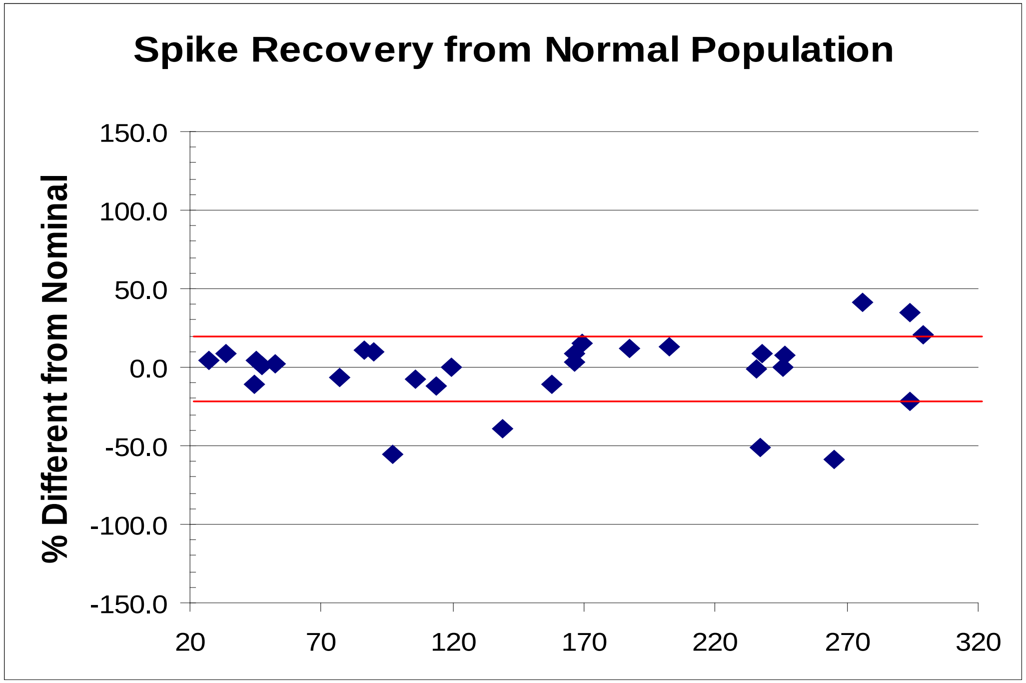
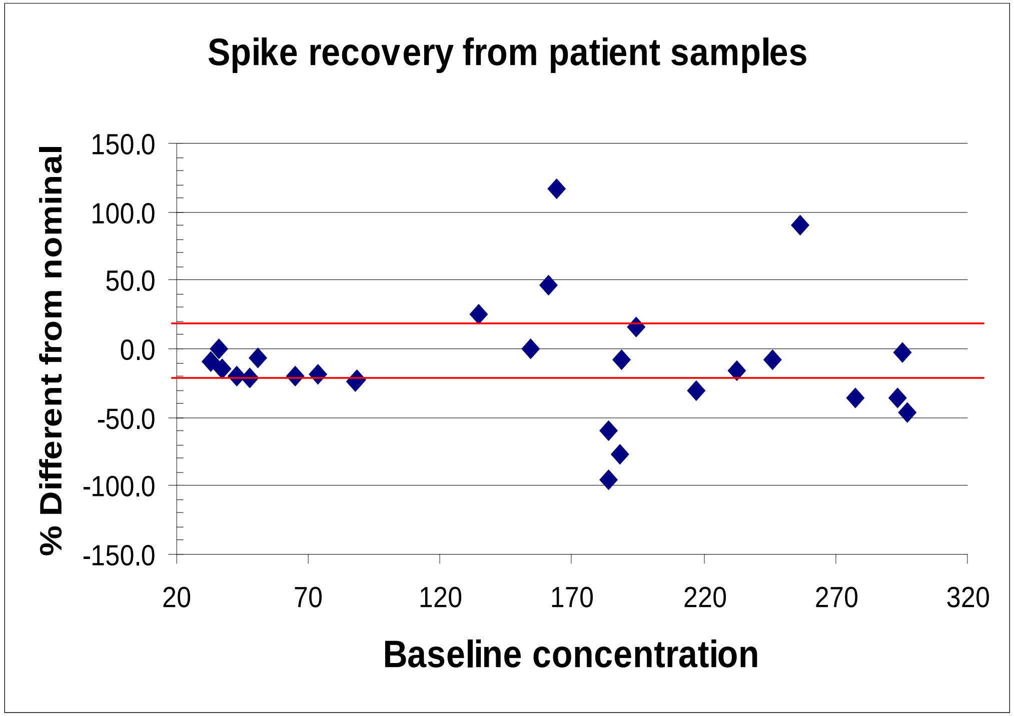
Supplemental Information



Another way of Parallelism Tests

Spike recovery of multiple lots from healthy normal and patients.



Spiking 150 mU

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