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A Biocon company

Bioanalytical strategy for Biosimilars: Recommended steps for establishment of comparability

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Head and Test Facility Management
Regulated Bioanalytical Laboratory

Topic for Discussion

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1. Brief Introduction: Clinigene International Limited & Regulated BA Laboratory
2. Assay Platforms, Compliance & Instrumentation
3. Large Molecules & Biosimilars- requirement for comparability assays
4. Efficacy & Safety: PK & ADA assays
5. Recent AAPS White paper on PK assay recommendations for Biosimilar development
    a. One/Two assay approach
    b. Method Development & Validation
    c. Balanced Assay Design
    d. Sample Analysis
6. Conclusion

Clinigene International Limited

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Clinigene

CLINICAL RESEARCH

Pioneer in customized quality Clinical Research solutions

150+ employees

- Clinical Trials
- Bioanalytical Research
- Clinical Data Management
- BA/ BE Testing

Clinigene is a 100% subsidiary of Syngene

Syngene

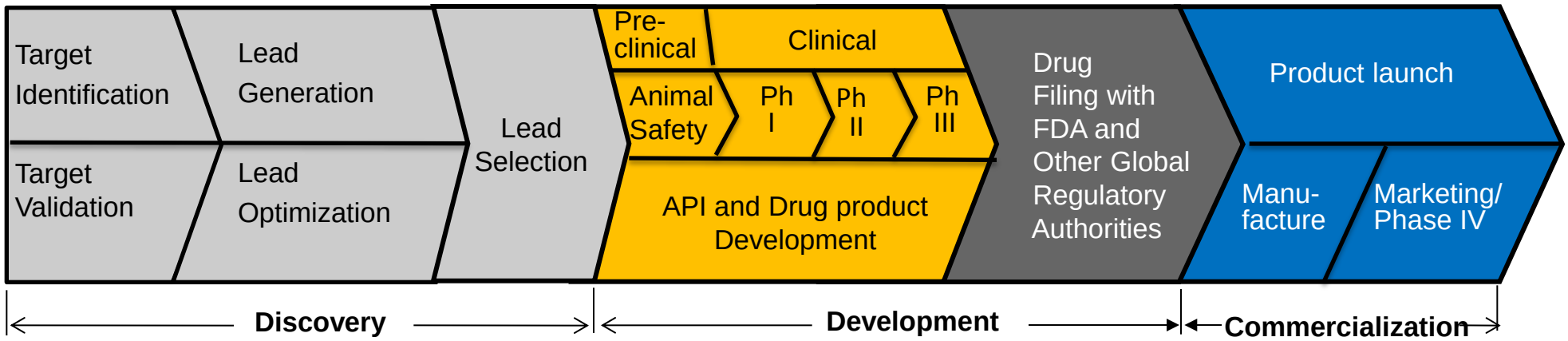
DISCOVERY & DEVELOPMENT

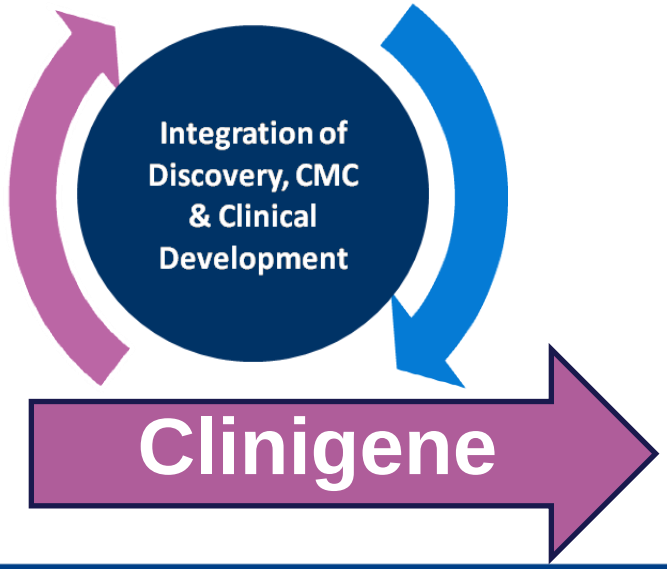
India's **largest** CRO

2150+ employees

- Chemistry
- Biology
- Toxicology (GLP/ non-GLP)
- API Manufacturing
- Formulation Development
- Analytical & Stability Studies
- Biologics Development

Syngene Clinigene:
Integrated Discovery & Development Platforms





Clinigene Services

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- Human Pharmacology Studies
- Bioanalytical & Immunoassay services
- Central Laboratory Services
- Clinical Trial Management
- Medical Writing
- Medical Monitoring & Safety Management
- EDC, Data Mgmt. & Biostatistics
- Regulatory Affairs & Pharmacovigilance
- Quality Assurance

Therapeutics

- Oncology
- Diabetology
- Rheumatology
- Metabolic disorders
- Dermatology
- Cardiovascular
- Gastroenterology
- Nephrology
- Immunology
- Infectious Diseases
- Neuropsychiatry

Products

- Small Molecules
- Biologics
- Biosimilars
- Vaccines
- Devices

Populations

- Neonatal
- Pediatric
- Adult

- Healthy volunteers
- Specific patient population

History of the Bioanalytical Laboratory

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2005- 2008	2008- 2011	2011-2014	2014 onwards
✓ Established as an Immunoassay Research Laboratory	✓ Implemented GLP and GCLP for system & compliance	✓ Turned into a full service CRO	✓ Imported and Analyzed >150,000
✓ Support development of Biologics & Biosimilars	✓ Adapted FDA/EMA regulations for technical compliance	✓ Supporting international submissions with GLP compliant data	✓ Business continuity plan tested
✓ Supported non clinical and clinical studies		✓ Added Small Molecule GLP laboratory	✓ Sample shipment and import licenses logistics well ironed
✓ Large Molecule PK, Immunogenicity , Neutralizing antibody assay, process related impurity assays		✓ Adapted 21 CFR Part 11 CSV principles	✓ Adapted STAR and WATSON LIMS for sample analysis and data reporting
		✓ Aligned with global clinical and nonclinical sites for multisite studies- sample manifests & data transfer formats	✓ Handling multiple clinical programs
		✓ Working with leading Indian & international biopharma	✓ Supporting PK, Biomarkers & Immunogenicity studies

Assay platforms & compliance

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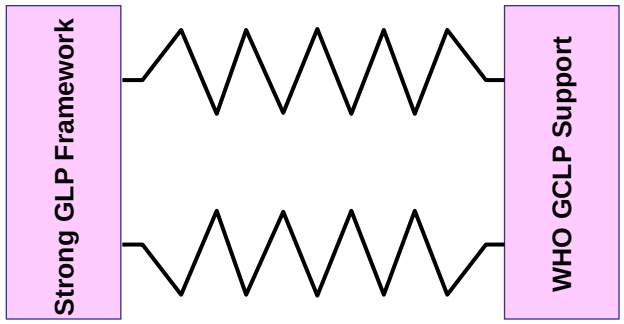
Assays in biological matrices

- Pharmacokinetic & 3 Tier Immunogenicity (ADA)
- Biomarkers
- Biosimilar- *analytical comparability*
- LC-MS/MS for NCEs and Generics

Technology platforms

- ELISA,, RIPA, RIA, SPR
- In Vitro Cell Based Functional assays
- MSD
- Potency assays
- LC-MS/MS



- ✓ All processes SOP driven
- ✓ All activities plan and report format
- ✓ Active role for the Test Facility Management
- ✓ Quality Assurance audit
- ✓ Defined TICO & archives
- ✓ Data quality, integrity, traceability & reliability
- ✓ Patient confidentiality & safety
- ✓ Expedited reporting

Assay Platforms & Instrumentation

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	Instrumentation	Validated	Numbers
• PK • Immunogenicity • Biomarker	MSD	☑	2
	ELISA plate readers	☑	4
	ELISA plate washers	☑	5
• Neutralizing antibody	Gamma & Beta Counters	☑	2 each
• Cell based assays	Cell Culture Laboratory	☑	1 lab
• Small Molecule Analysis	LC-MS/MS	☑	2
	Centrifuge (Tube & Plate)	☑	2
• Other equipment	Tecan automation	☑	2
	Incubators	☑	7
	Freezers	☑	12

Repairs: **Average Equipment repair turnaround Time: 24-48 Hours for Minor; and 1-2 weeks for Major**

Assay Platforms: Unique features

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Technique	Nomenclature	Applied in	Advantages
ELISA (Non Radioactive)	Includes ACE & bridging ELISA	Immunogenicity, PK, TK	Multiple formats: Bridging and Sandwich
Radioactive Immunoassays	Includes RIPA & RIA **Proliferation/ Uptake assays**	Immunogenicity, PK, TK	Higher Sensitivity and Specificity
Functional Cell Based assays (Radioactive & Non Radioactive)	Potency & Neutralizing Ab (Nab) **Efflux Assays**	Immunogenicity, Potency	Functional Assays to evaluate Bioactivity and presence of NAb
Surface Plasmon Resonance Assays	Ligand Analyte relationship AB Characterization	Immunogenicity, Affinity and Ligand Binding Kinetics	Real time, Low volumes & no washing steps
Electrochemiluminescent Immunoassay	MSD **Multiplexing**	Immunogenicity, PK, TK	Lower volumes, higher throughput & Drug Tolerance

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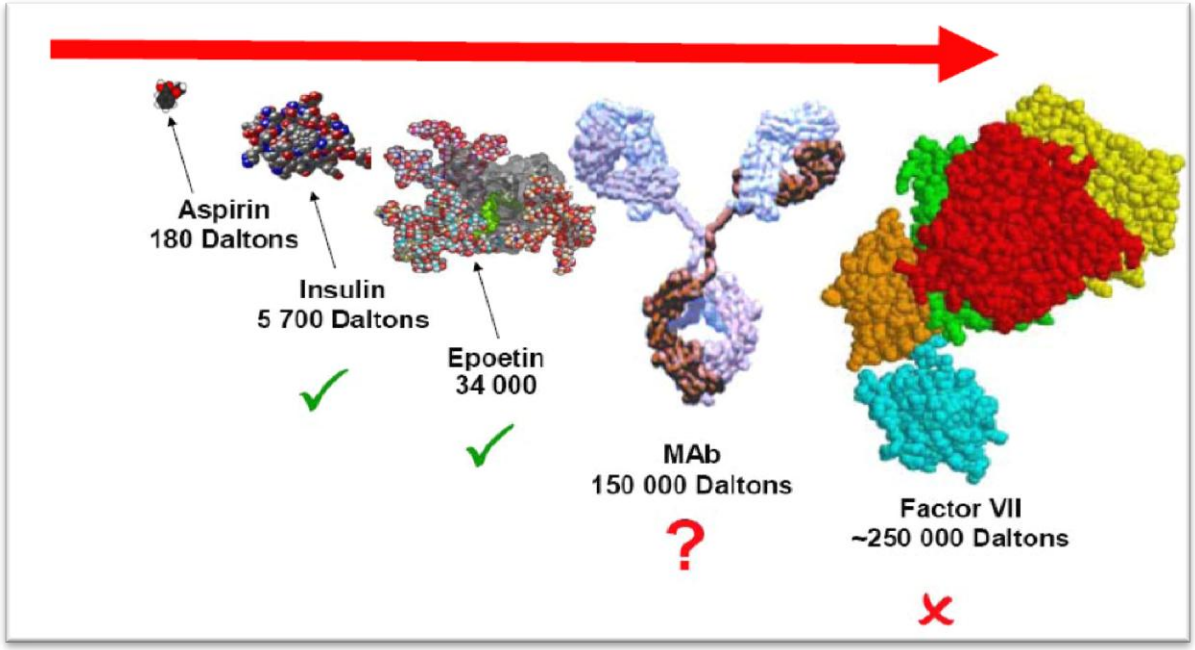
Large Molecules also called Biologics = >3 KD? With a complex structure

- Biological products are medicines derived from natural sources like microorganisms, animal & humans.

- Owing to the scientific and technical complexities that may be associated with these larger and often more complex structure of biological products, as well as the processes by which such products are manufactured, the Bioanalysis for these products prove to be particularly challenging.

Biologics (Large Molecules = >3 KD?)

- Proteins
- Peptides
- Fusion proteins
- Monoclonal Antibodies



How do Large Molecules (Biologics) differ from Conventional drugs

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Biological products

- Wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins.
- Can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues.
- Isolated from a variety of natural sources - human, animal, or microorganism –
- May be produced by biotechnology methods
- May be used to treat a variety of medical conditions for which no other treatments are available.

How do biological products differ from conventional drugs?

- Most SM drugs are chemically synthesized and their structure is known, most biologics are complex mixtures that are not easily identified or characterized.
- Biological products, including those manufactured by biotechnology, tend to be heat sensitive and susceptible to microbial contamination.
- Necessary to use aseptic principles from initial manufacturing steps, which is also in contrast to most conventional drugs. (GMP)
- Biological products often represent the cutting-edge of biomedical research
- In time, may offer the most effective means to treat a variety of medical illnesses and conditions that presently have no other treatments available.

Complexity of Biologics and International Guidance Documents

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U.S. Food and Drug Administration

Protecting and Promoting *Your* Health

- Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (PDF - 140KB)
- Draft: Points to Consider in the Design and Implementation of Field Trials for Blood Grouping Reagens and Anti-Human Globulin (PDF - 211KB)
- Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (PDF - 279KB)
- Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (PDF - 30KB)
- Draft of Points to Consider in the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Antibodies to the Human Immunodeficiency Virus, Type 1 (PDF - 1.7MB)
- Points to Consider in the Collection, Processing, and Testing of Ex-Vivo Activated Mononuclear Leukocytes for Administration to Humans (PDF - 208KB)
- Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologic & Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability (PDF - 107KB)
- Interferon Test Procedures: Points to Consider in the Production and Testing of Interferon Intended for Investigational Use in Humans (PDF - 545KB)
- Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals (PDF - 182KB)

EUROPEAN MEDICINES AGENCY

SCIENCE MEDICINES HEALTH

Quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological / biological products	Adopted guideline
Position statement on the use of tumorigenic cells of human origin for the production of biological and biotechnological medicinal products	Adopted guideline
Position statement on DNA and host cell protein impurities, routine testing versus validation studies	Adopted guideline
Allergen products: Production and quality issues	Overview of comments Adopted guideline Draft guideline
Quality of biotechnological products: Analysis of the expression construct in cell lines used for production of rDNA-derived protein products	Adopted guideline
Production and quality control of medicinal products derived by recombinant DNA technology	Adopted guideline
Gene therapy product quality aspects in the production of vectors and genetically modified somatic cells	Adopted guideline

Biosimilars

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- ✓ A **Biosimilar** is a similar version of the active substance of a biological therapeutic -Reference or Innovator product
- ✓ Biosimilar must demonstrate **similarity** to the Reference biological therapeutic in physicochemical characteristics, pharmacokinetics, efficacy and safety.
- ✓ Biosimilars /**biological products**: peptide therapeutics, monoclonal antibodies, receptor fusion proteins & endogenous proteins
- ✓ Owing to **unavoidable differences** in the manufacturing processes, the quality attributes of the Biosimilar and the Reference product are not expected to be entirely identical
- ✓ Any differences found have to be explained and **justified with regard to the impact on the safety and efficacy of the Biosimilar**
- ✓ The interest in Biosimilar -increasing population of patients with an **acute need for affordable high quality biologics**,
- ✓ **Guidance documents** for the development and manufacturing of Biosimilars have been published by EMA & FDA for comparability protocols in support of CMC (Chemistry, Manufacturing & Controls), non-clinical and clinical studies
- ✓ There is a **lack of specific regulatory guidance** around the requirements for the Bioanalytical testing of Biosimilars and Reference biological products in comparability studies that support the development of Biosimilars.
- ✓ Good quality data hinges on **best practices** & careful, intelligent decisions taken for the most **unbiased evaluation** of safety & efficacy
- ✓ Comparable PK and Immunogenicity assays are judged to be critical for a Biosimilar study

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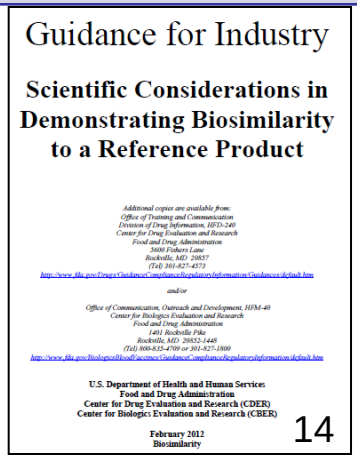
Requirement	What does this mean	
Analytical Studies	Extensive structural & functional characterization at CMC stage	• Primary Structures • Higher order structures • Post-translational modifications • Other Potential Variants- deamidation/oxidation • Intentional Chemical modifications- Pegylation etc.
Animal Studies	Preclinical studies in pharmacologically relevant animal models	• Animal Toxicity studies • Animal PK & PD measurements • Animal Immunogenicity studies
Clinical Studies	Clinical studies in relevant ethnic population	• Demonstration of Safety, Purity & Potency • PK & PD measurements • Assessment of Immunogenicity

Demonstration of Comparability

Use of *Totality-of-the-Evidence* Approach to Assess a Demonstration of Biosimilarity between the Innovator and Comparator


Guidance for Industry

Scientific Considerations in Demonstrating Biosimilarity to a Reference Product

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

February 2012
Biosimilarity

Safety & Efficacy: PK and ADA Assays

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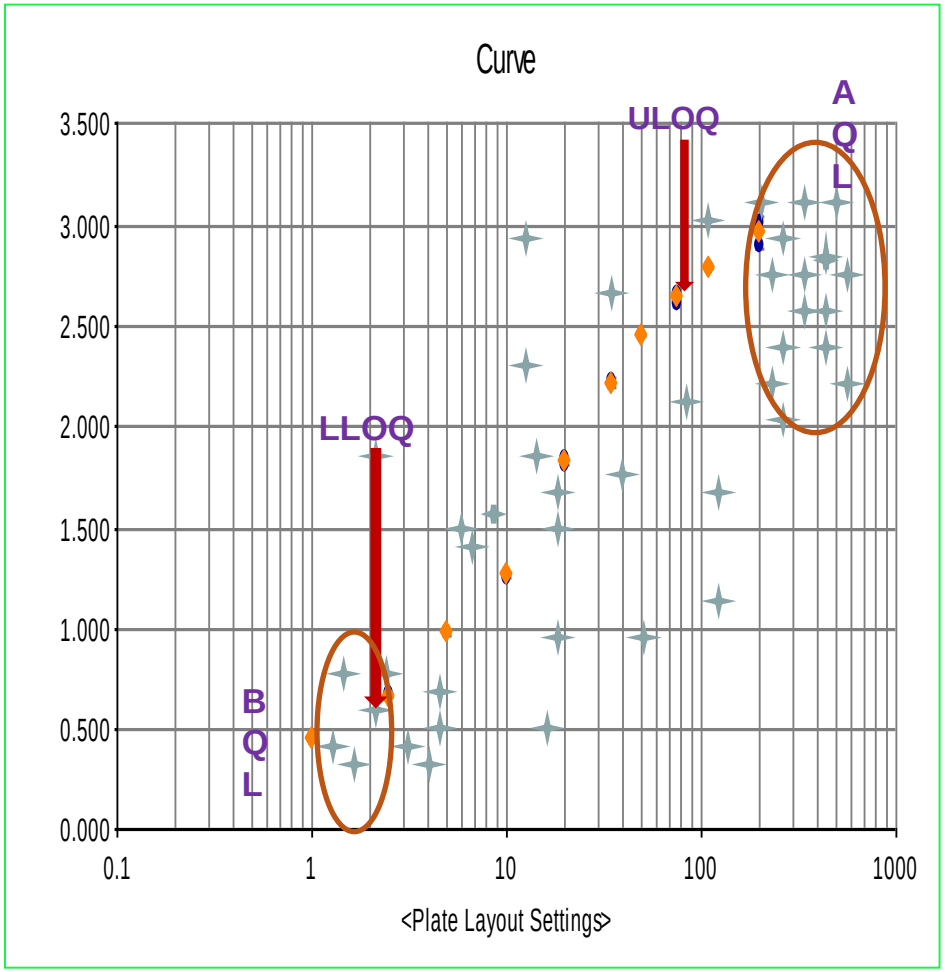
Blinded Clinical Samples

Sample Pre assessment for Probable dilution

AQL: Above Quantification Limits- Reanalysis

BQL: Below Quantification Limits- Reanalysis



Potential ADA samples

-ve Samples rejected

Screening Assays

+ve Samples

Specificity/Confirmatory Assay

Neutralization Assay

Confirmed +ve Samples

Titre & Charactn

Assess & Report correlation of characterized antibodies with clinical responses to biologic therapeutic

AAPS Committee Recommendation White Paper

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The AAPS Journal (© 2014)
DOI: 10.1208/s12248-014-9669-5

White Paper

Systematic Verification of Bioanalytical Similarity Between a Biosimilar and a Reference Biotherapeutic: Committee Recommendations for the Development and Validation of a Single Ligand-Binding Assay to Support Pharmacokinetic Assessments

Joseph C. Marini,[1,11] Michael Anderson,[2] Xiao-Yan Cai,[3] John Chappell,[4] Todd Coffey,[5] Dominique Gouty,[6] Aparna Kasinath,[7] Vera Koppenburg,[8] Philip Oldfield,[9] Shannon Rebarchak,[1] and Ronald R. Bowsher[10]

Received 21 April 2014; accepted 29 June 2014

What	APC consensus for a systematic and statistics based process to confirm bioanalytical similarity
Goal	Use of a single LBA method to support PK/TK assessments of both a Biosimilar and a Reference therapeutic
Who	Investigators who are conducting 'Regulatory-Compliant' bioanalysis
Why	A single LBA method is more operationally practical, reduces the variability of the data related to potential assay differences and allows the bioanalytical scientist and clinical pharmacologist to remain blinded to dosing.
When	For support of non-clinical and clinical studies

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DOI 10.1208/s12248-014-9669-5

*There is a **lack of specific regulatory guidance** around the requirements for the Bioanalytical testing of Biosimilars and Reference biological products in comparability studies that support the development of Biosimilars*

One Assay or Two Assays

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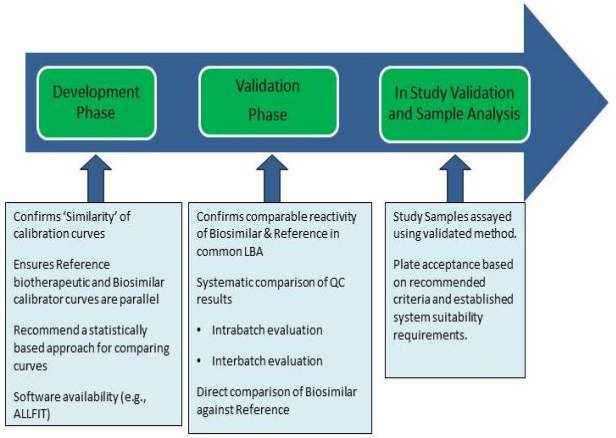
One-assay approach		Two-assay approach	
Pro	**Con**	**Pro**	**Con**
Conservative approach to use biosimilar curve for quantification of both Biosimilar and Reference drug concentrations.	Need to demonstrate minimal absolute difference in %RE between Biosimilar QCs and Reference QCs.	Concentration of Biosimilar and Reference drug will be calculated from curve of same respective material, eliminating variability due to differences between curve material and QC material.	Need to perform cross comparison of QCs against both curves to establish a true comparison between QCs that would demonstrate bioanalytical similarity.
No 'between-assay' variability, i.e. minimization of the potential impact of assay bias on the comparison of the biosimilar and the reference product.			Two assays with different properties: different reagents, assay characteristics (selectivity, sensitivity, etc.). • Introduction of additional variability that might reduce the reliability of the comparison.
Blinded study sample analysis possible.			Blinded analysis would require all samples to be run through both assays.
Need to develop and validate one assay.			Need to develop and validate two assays.



Method Development & Validation

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Assay Parameters *(recommended at minimum)*	Phase of Study	Status
• Similarity of assay calibration curves and quality control samples • Establish target acceptance criteria for MV	**Method Development**	☑☒
• Assay Methodology/ Platform • Critical Reagents- drug specific • Assay Design: Calibrators & QCs • Assay Calibrators: Reference (US/EU) or Biosimilar	**Method Development**	☑☒
• Accuracy & Precision- Inter & Intra Batch • Selectivity • Dilutional Linearity • Stability	**Method Validation**	☑☒
• Comparative statistics to assess bioanalytical similarity • Mathematically derived systematic verification	**Method Development & Method Validation**	☑☒

Recommended Balanced Assay Designs for PK evaluation of Biosimilar and Reference

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Method Development:
Use an assay design that minimizes potential experimental bias in positional, operator, and/or environmental effects

Plate 1 — Biosimilar — Reference

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1
B	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2
C	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3
D	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4
E	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5
F	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6
G	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7
H	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8	Std 8

Method Validation
The QC samples from the Biosimilar and Reference should be evaluated against a single calibration curve

Plate 1 — Biosimilar — Reference

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9	Std 10	Std 11	Std 12
B	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9	Std 10	Std 11	Std 12
C												
D	ULOQ-1a	ULOQ -1b	ULOQ-1a	ULOQ -1b	ULOQ-2a	ULOQ-2b	ULOQ-2a	ULOQ-2b	ULOQ-3a	ULOQ-3b	ULOQ-3a	ULOQ-3b
E	HQC-1a	HQC-1b	HQC-1a	HQC-1b	HQC-2a	HQC-2b	HQC-2a	HQC-2b	HQC-3a	HQC-3b	HQC-3a	HQC-3b
F	MQC-1a	MQC-1b	MQC-1a	MQC-1b	MQC-2a	MQC-2b	MQC-2a	MQC-2b	MQC-3a	MQC-3b	MQC-3a	MQC-3b
G	LQC-1a	LQC-1b	LQC-1a	LQC-1b	LQC-2a	LQC-2b	LQC-2a	LQC-2b	LQC-3a	LQC-3b	LQC-3a	LQC-3b
H	LLOQ-1a	LLOQ-1b	LLOQ-1a	LLOQ-1b	LLOQ-2a	LLOQ-2b	LLOQ-2a	LLOQ-2b	LLOQ-3a	LLOQ-3b	LLOQ-3a	LLOQ-3b

Sample Analysis

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- ✓ A validated method should be used for Sample Analysis
- ✓ All assay plates should include the appropriate calibrator selected during the development phase
- ✓ At minimum all plates should contain H,M, & L QCs using appropriate drugs
- ✓ All other criteria should be determined by validation and trial requirements

It is recommended to base all assay acceptance criteria for validation and sample analysis on established industry standards and Guidance documents

Conclusion

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- ✓ Systematic process for assessing degree of BA similarity between a Biosimilar & Reference drug
- ✓ 2 staged approach consisting of development and validation phases
- ✓ Data from above to support use of one or two assays for quantification of a Biosimilar and Reference drug in a head to head trial
- ✓ Caution and Attention!! **A critical assumption for use of a common calibrator is that the Biosimilar and Reference Biotherapeutics have undergone detailed characterization and have previously been judged to be comparable with respect to their physicochemical and biological characterization**

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Questions & Discussion

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Back up slides

Critical Reagent

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