Quotient Bio Analytical Sciences
an LGC business

A Global view on parallelism

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Overview

- Definition
- Literature on parallelism
- Global Regulatory guidance
- Global Bioanalysis Consortium
- GBC L2 Harmonisation Team
- Summary

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- **Definition**
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Definition

Parallelism -"A condition in which dilution of test samples does not result in biased measurements of the analyte concentration"

- It is **not** dilutional linearity
- But the experimental design is similar

"Uses incurred samples to demonstrate that the sample-dilution response curve is parallel to the standard-concentration response curve"

- It is **not** a method issue
- It **is** a sample issue

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Literature

Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules

Binodh **DeSilva**, Wendell Smith,Russell Weiner,Marian Kelley, JoMarie Smolec, Ben Lee, Masood Khan, Richard Tacey, Howard Hill and Abbie Celniker. *Pharmaceutical Research, Vol. 20, No. 11, November 2003 (© 2003)*

"Parallelism is a performance characteristic that is typically evaluated during in-study validation. It is conceptually similar to dilutional linearity except that it is assessed with multiple dilutions of actual study samples or samples that represent the same matrix and analyte combination as those that will be generated during a study"

- Method development - not typically evaluated
- Pre-study validation - evaluate where-ever possible
- In-study validation - 'can' be assessed using pooled Cmax samples. As a target, it is recommended that the relative standard deviation (%CV) between samples in a dilution series be 30%. The procedure for reporting a result when a sample does not dilute linearly (i.e., non-parallel) should be defined *a priori*.

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Regulatory guidance - EMA

EMA guideline on bioanalytical method validation, 2011

- **7. Ligand binding assays**
- **7.1 *Method validation***
  - **Full validation**
    - ***7.1.1.10 Parallelism***
- If study samples are available, parallelism between the calibration standard curve and serially diluted study samples should be assessed to detect possible matrix effect or differing affinities for metabolites. A high concentration study sample (preferably close to Cmax) should be diluted to at least three concentrations with blank matrix. The precision between samples in a dilution series should not exceed 30%.

- In case the sample does not dilute linearly (i.e. in a non parallel manner), a procedure for reporting a result should be defined a priori.

- If study samples are not available during the validation of the method, parallelism should be evaluated as soon as study samples become available.

Regulatory guidance - FDA

FDA Guidance on Bioanalytical Method Validation, 2001

- V. **METHOD DEVELOPMENT: MICROBIOLOGICAL AND LIGAND-BINDING ASSAYS**
  - **A. Selectivity Issues**
    - *2. Matrix effects Unrelated to the Analyte*
  - Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects.

Regulatory guidance - FDA

DRAFT FDA Guidance on Bioanalytical Method Validation, 2013

- **IV. LIGAND BINDING ASSAYS**
  - **B. Bioanalytical Method Development and Validation**
    - b. Matrix Effects
  - Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects.

Other regulatory agencies

- **Japan MHLW** - Guideline on Bioanalytical Method Validation in Pharmaceutical Development
  - Focuses on LC, GC and MS (not LBA)
  - **"***A draft guideline on LBAs is expected to be issued towards the end of March 2014.***"** Proposed by the Japan Bioanalysis Forum (JBF) that parallelism is included

- **Brazil ANVISA -** Similar to EMA, but no specific mention of LBA, recommendation is to apply if possible
- **Canada TPD -** Similar to EMA, follow EMA for LBA
- **China SFDA -** Similar to EMA, but no specific mention of LBA

Other groups

- TT-35 within the EBF

- Global CRO Council (GCC)

Recommendations on the interpretation of the new European Medicines Agency Guideline on Bioanalytical Method Validation by Global CRO Council for Bioanalysis (GCC)

"These North American and European events provided a unique opportunity for CRO leaders to openly share opinions and perspectives and to agree on unified bioanalytical recommendations specifically in relation with the new EMA guideline."

Mark Boterman[1], Mira Doig[2], Massimo Breda[3], Steve Lowes[4], Jim Jersey[5], Ronald Shoup[6], Fabio Garofolo[7], Isabelle Dumont[7], Suzanne Martinez[7], Shane Needham[8], Maria Cruz Caturla[9], Philippe Couerbe[10], Joelle Guittard[11], John Maltas[12], Tim Lansing[13], Masood Bhatti[14], Christine Schiebl[15], Petra Struwe[15], Curtis Sheldon[16], Roger Hayes[17], Timothy Sangster[18], Colin Pattison[18], Johanne Bouchard[19], Lee Goodwin[20], Rafiq Islam[21], Rudi Segers[22], Zhongping (John) Lin[23], Jim Hillier[24], Wei Garofolo*[25], Dieter Zimmer[26], Lois Folguera[27], Thomas Zimmermann[27], Maria Pawula[28], Marc Moussallie[29], Leonardo de Souza Teixeira[30], Thais Rocha[30], Daniel Tang[31], Paula Jardieu[32], James Truog[33], Jenny Lin[33], Richard Lundberg[34], Chris Cox[35], Alan Breau[36], Chiara Bigogno[37], Dick Schoutsen[38], Carmen Dilger[39], Mohammed Bouhajib[40], Ann Levesque[41], Sofi Gagnon-Carignan[41], Robert Nicholson[42], Rand Jenkins[42], Ming Hung Lin[43], Shane Karnik[44], Theo De Boer[45], Richard Houghton[46], Rachel Green[46], William DeMaio[47], Romuald Sable[48], Kirk Smith[49], Christoph Siethoff[50], Laura Cojocaru[51], Mike Allen[52], Tammy Harter[53], Saadya Fatmi[54], Farhad Sayyarpour[55], Michele Malone[56], Stuart Best[57] & Xinping Fang[58]

Note that due to equality principals of Global CRO Council (GCC), authors are presented in alphabetical order of company affiliation.

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Bioanalysis (2012) 4(6), 651–660

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GLOBAL CRO COUNCIL

Bringing many CRO leaders together
to openly discuss bioanalysis and the regulatory challenges
unique to the outsourcing industry

Box 18. The GCC recommendation on parallelism.

- The GCC recommends that parallelism should be performed with incurred samples, but care should be taken when selecting the incurred sample with respect to possible metabolites, excipients and so on. If these experiments are performed within a sample analysis study the results should be reported within the bioanalytical report. It is also recommended that the aspects of Good Clinical Practice should be considered. For incurred samples to be used for parallelism evaluations this would need to be included in the clinical protocol and in the informed consent. For pre-clinical studies, the experiment should be included in the toxicology protocol.

- Global Bioanalysis Consortium (GBC)

Global Bioanalysis Consortium
On harmonization of bioanalytical guidance

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Global Bioanalysis Consortium

Mission Statement

Create an all inclusive **Global Bioanalysis Consortium** (GBC) consisting of represented **scientific associations** with world wide influence to merge existing or emerging bioanalytical guidance to create one, **unified consensus document** that can be presented to the regulatory bodies/health authorities in various countries.

http://www.globalbioanalysisconsortium.org/

Global Bioanalysis Consortium

On harmonization of bioanalytical guidance

Global Bioanalysis Consortium

Goals and Objectives:

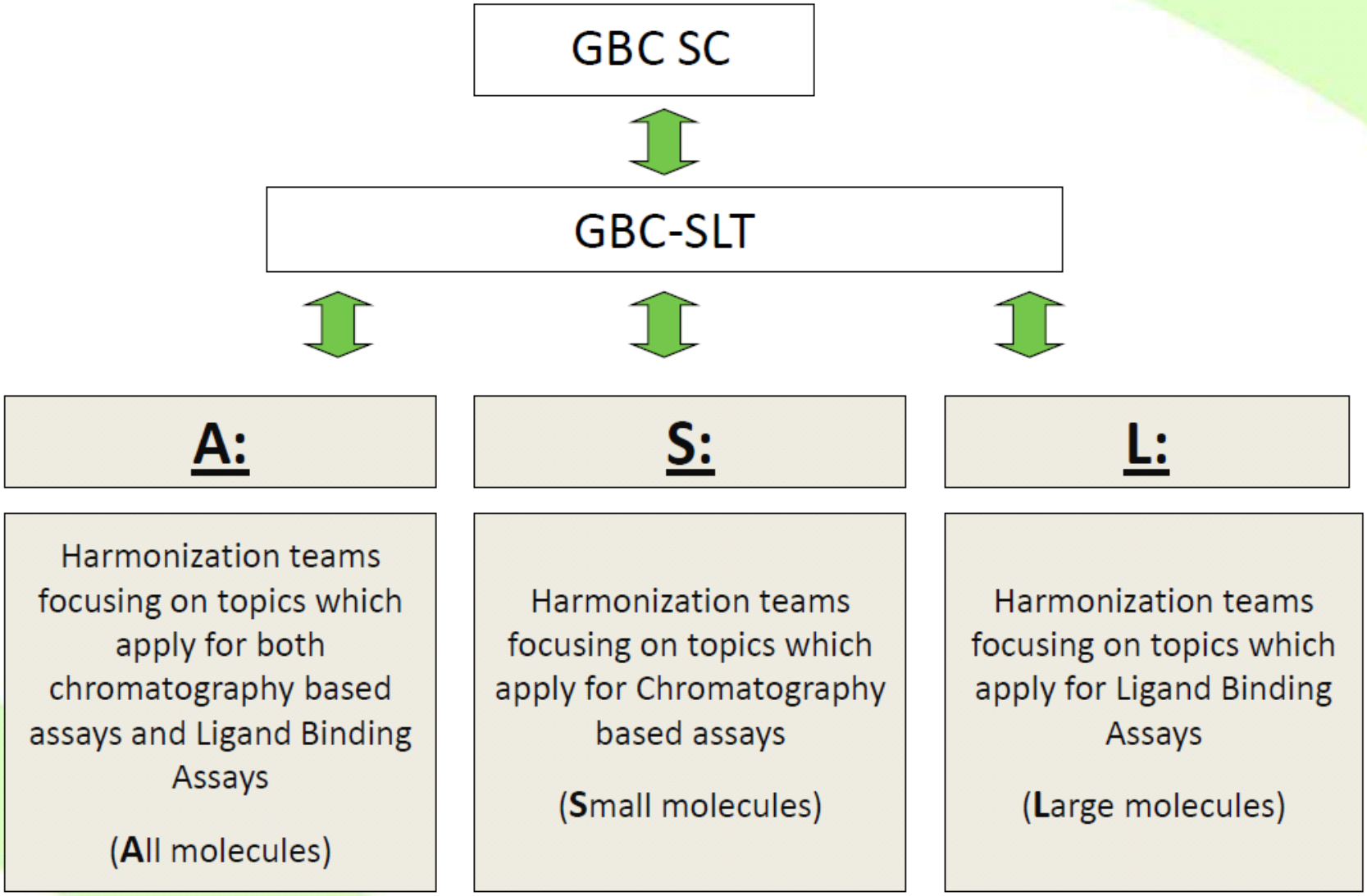
To bring together stakeholders from the **pharmaceutical industry, contract research organizations and academia** to **share** current understanding of bioanalysis guidelines, identify differences in these guidelines or differences in the interpretation or application thereof to routine regulated bioanalysis.

To come forward with **recommendations** to Health Authorities and regulatory bodies worldwide on globally agreed **best practices** for Bioanalytical Method Validation (BMV) and application of such methods/technologies to the analysis of drugs of all molecular sizes in support of clinical and nonclinical studies.

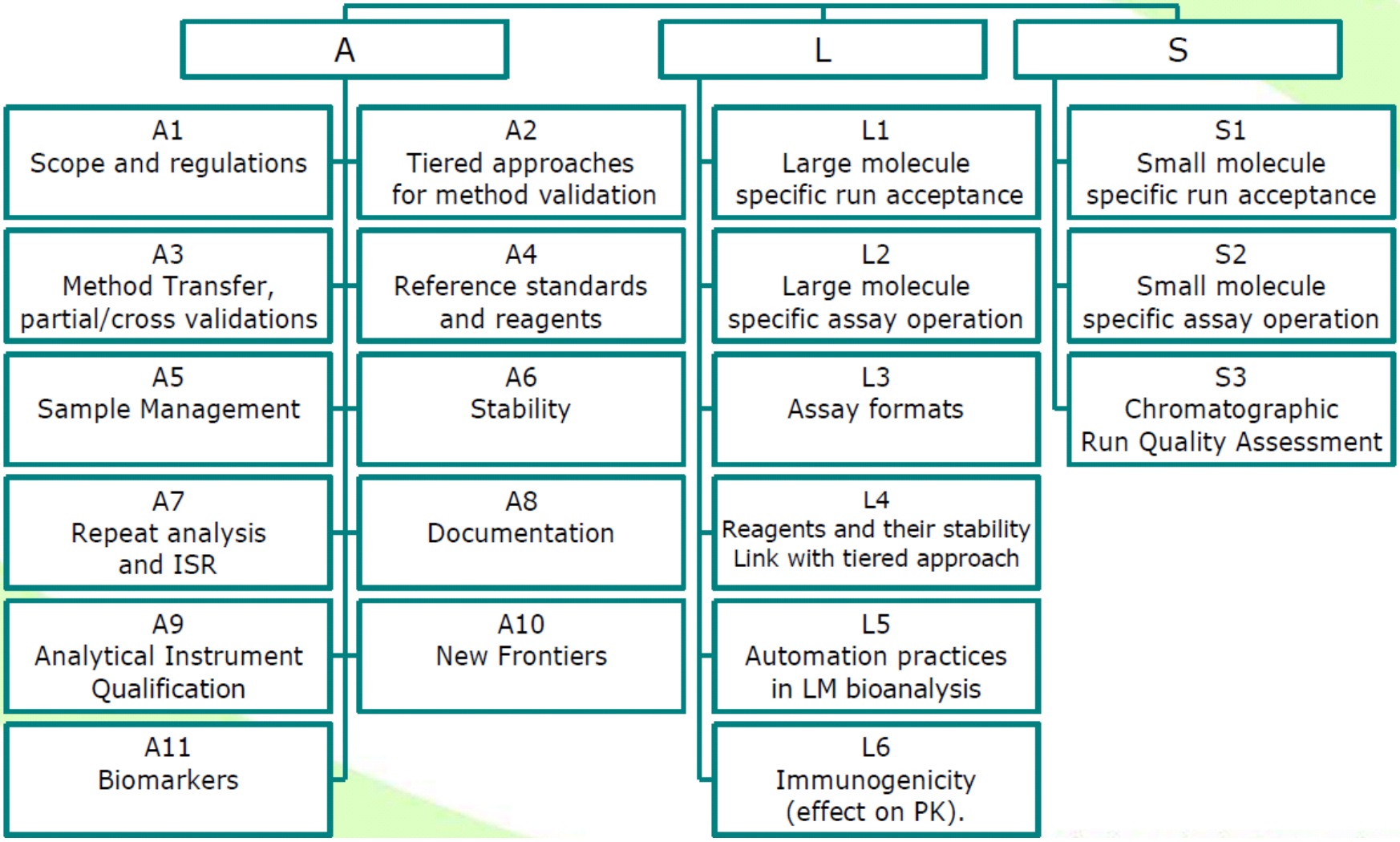
Global Bioanalysis Consortium

On harmonization of bioanalytical guidance

GBC – Organizational structure



GBC - Harmonization teams



GBC - Harmonization teams- The L teams

L1 Marian Kelley

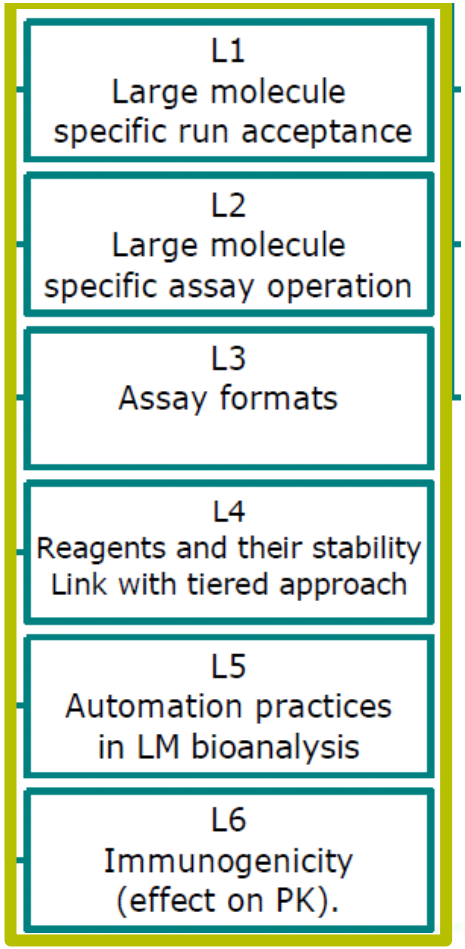
L2 Lauren Stevenson

L3 Sherri Dudal

L4 Lindsay King

L5 Scott Davis

L6 Jeff Sailstad



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L2 Large Molecule Specific Assay Operation

Team members:

Team lead

– Lauren Stevenson (NA)

Other members

– Clare Kinglsey (EU)
– Karolina Oesterlund (EU)
– Marian Kelley (NA)
– Heather Myler (NA)
– Boris Gorovits (NA)
– Yoshiyuki Minamide (APAC, Japan)
– Arumugam Muruganandam (APAC, India)
– Mario Dominguez (LA)

In scope

- Testing of ruggedness and robustness
- Setting up a balanced validation design
- Dilution linearity
- Specificity testing
- Selectivity testing
- Parallelism
- Hook effect

Interdependencies with other teams

– L1 – Assay Acceptance
– A6 – Stability

Out of scope

- Cross validation (A3)
- Approach for spiking QCs for validation (L1)
- Use of drug product, drug substance or reference standard as the entity used in validation/sample analysis (A4)
- Long term stability in matrix (A6)

L2 Team output

"Large Molecule Specific Assay Operation: Recommendation for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team"

Accepted for publication on 15 Oct 2013 in the AAPS Journal

Online before Crystal V (December 2013)

Stevenson, Lauren, Kelley, Marian, Gorovits, Boris, Kingsley, Clare, Myler, Heather, Österlund, Karolina, Muruganandam, Arumugam, Minamide, Yoshiyuki, Dominguez, Mario

Abstract

The L2 Global Harmonization Team on large molecule specific assay operation for protein bioanalysis in support of pharmacokinetics focused on the following topics: setting up a balanced validation design, specificity testing, selectivity testing, dilutional linearity, hook effect, parallelism, and testing of robustness and ruggedness. The team additionally considered the impact of lipemia, hemolysis and the presence of endogenous analyte on selectivity assessments as well as the occurrence of hook effect in study samples when no hook effect had been observed during pre-study validation.

Keywords

Selectivity; specificity; parallelism; lipemic samples; hemolyzed samples; robustness, ruggedness; hook effect; in-study hook effect

L2 HT- Parallelism- Current status

- The concept of parallelism is similar to dilutional linearity except that parallelism assesses incurred study samples

- At present, routine parallelism assessments are **not being broadly implemented industry-wide**

- Non-parallelism examples were:
  - rare
  - involved non-mAb therapeutics
  - became apparent during scientific review of the sample data

L2 HT- Parallelism- When should it be included?

- Routine parallelism assessments are ***not recommended***

- ***Scientific rationale*** should drive the decision to perform parallelism assessment and this should be dependent on the nature of the therapeutic

- Factors to consider:
  - propensity of the drug to aggregate
  - drug stability in vivo (eg partial degradation or biotransformation)
  - presence of anti-drug antibodies
  - endogenous binding partners
  - assay specificity toward complexes eg free vs bound forms

L2 HT- Parallelism- How to include it?

- Although parallelism has been mentioned for PK assays since 2003 (DeSilva et al) no consensus for testing has emerged

- By definition, it cannot be performed until incurred study samples become available
  - Therefore is covered as in-study validation
  - Often once a problem with PK profiles has been identified

- Testing requires multiple dilutions within the assay range
- Inter-dilution precision (%CV) criterion of less than 30% should be applied
- Pooled or individual samples can be used
- General advice is to test samples from later time points

L2 HT- Non-parallelism- What do you do?

- "Should non-parallelism be detected, a sample testing scheme will need to be put in place to mitigate the issue and an ***a priori* strategy** for data reporting will need to be established"
  - In some cases, simply increasing the dilution at which samples are tested may be adequate to drive dissociation of complexes causing the non-parallelism
  - In others, an alternative PK assay design may be required
- Is it always this clear cut?
  - It is also important to be aware that even when a parallelism assessment passes proposed acceptance criteria, **trends that may have meaningful impact** on the study data may still be present

L2 HT- Parallelism- A case study I

Sample ID	Sample dilution	Reportable result	Statistics	
Animal 1 Day 29	100	>ULOQ		
	2000	23969		
	3000	30991		
	4500	35237		
	6750	37398		
	10125	<LLOQ	SV	5919
	15188	<LLOQ	Mean	31899
	22781	<LLOQ	**CV%**	**18.6**
Animal 1 Day 57	100	>ULOQ		
	1000	19867		
	1500	24059		
	2250	32227		
	3375	28331		
	5063	32830	SV	5468
	7594	33255	Mean	28428
	11390	<LLOQ	**CV%**	**19.2**
Animal 2 Day 10	20	563		
	30	598		
	45	566		
	68	608		
	101	652		
	152	625	SV	35.8
	228	629	Mean	613
	342	660	**CV%**	**5.9**
Animal 2 Day 14	20	165		
	30	210		
	45	243		
	68	273		
	101	329		
	152	293	SV	61.0
	228	235	Mean	237
	342	152	**CV%**	**25.7**

L2 HT- Parallelism- A case study II

Sample ID	Sample dilution	Reportable result	Statistics	
Animal 1 Day 29	100	>ULOQ		
	2000	23969		
	3000	30991		
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	6750	37398		
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L2 HT- Parallelism- A case study III

- For all samples tested, the inter-dilution precision (%CV) is **well within the commonly applied ±30% criterion**

- However, the data suggest a **trend of increasing drug recovery with increasing dilution** for the samples collected from Animal 1 on days 29 and 57 and Animal 2 on day 14

- A **scientific judgment** should therefore be applied to determine whether an additional investigation is warranted

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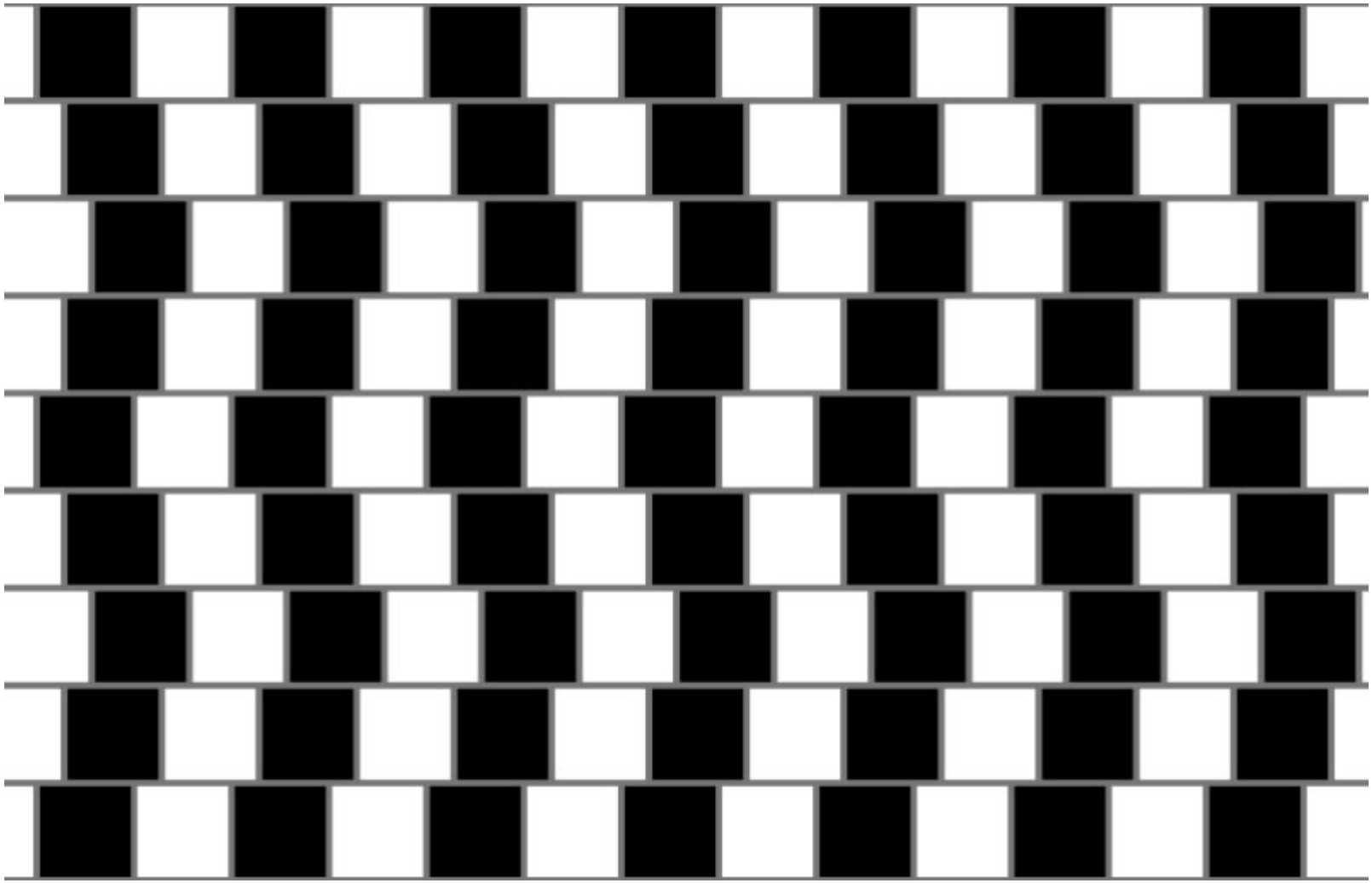
Parallelism - Is it real? Or an optical illusion?



Parallel Lines Cafe Wall Illusion
http://www.sciencekids.co.nz/pictures/illusions/parallellines.html

Summary

- Parallelism is a important issue in Ligand Binding Assays
  - The recommendations may be different for PK assays vs biomarkers
  - Parallelism is mentioned in the FDA guidance from 2001 and discussed in more detail in 2003
  - Some regulatory guidance remains very limited with respect to LBA
  - EMA offers the clearest guidance
  - Everything that is covered in current regulatory is based on this 2003 paper

Summary

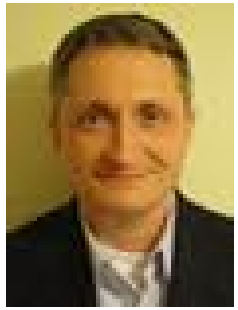
- The Global Bioanalysis Consortium L2 Harmonization team views are represented
  - The team does not recommend routine parallelism assessment
  - A rationale for testing should be based on an understanding of the biology
  - Non-parallelism is *rare*
  - Assessment should be via multiple dilutions of incurred samples and an inter-dilutional precision measurement
  - Careful review of study data can often reveal a non-parallelism issue
  - Even if acceptance criteria are met-

    take a close look at your data

Acknowledgements

- A special thanks to Lauren Stevenson, who lead the L2 HT effort
- Thanks to the L2 HT team members
  - Marian Kelley
  - Boris Gorovits
  - Heather Myler
  - Karolina Österlund
  - Arumugam Muruganandam
  - Yoshiyuki Minamide
  - Mario Dominguez






- TT-35 Parallelism team at the EBF
- EBF Open Symposium Committee

THANK-YOU

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Sciences
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