Validation of immunoassays: the importance of parallelism

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on behalf of EBF TT35

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Topic Team 35

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Content

- Background TT35
- Parallelism testing: why?
  - How to assess Parallelism?
  - What to do when Parallelism fails?
- TT 35 Draft Recommendation

Background TT35

- Increased application of Ligand Binding Assays to support Drug development programs
- Therapeutic and/or regulatory decision-making requires demonstration of validity of assay performance
- Implementation and routine assessment of parallelism seems still to be at its infancy

- ***Parallelism:***
  - ***What is it?***
  - ***How to assess?***
  - ***What to do if it fails?***

Approach TT35

- Survey was sent out to all EBF-IGM members
  - 11 # 34 received

- Survey outcome did not provide (enough) consensus on establishing a general procedure (recommendation) for parallelism assessment of Biotherapeutic and/or Biomarker quantitative assays

- Team members each represented (slightly) different procedures for assessment of parallelism, depending on:
  - Biotherapeutic or Biomarker quantification
  - Biology
  - Purpose

- AIM: Create awareness!
  - Collect case studies

Definition: Parallelism

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

Thus, when a test sample is serially diluted to result in a set of samples having analyte concentrations that fall within the quantitative range of the assay, there is no apparent trend toward increasing or decreasing estimates of analyte concentrations over the range of dilutions."

(Miller et al., Pharm Research 18(9), 1373-1381, 2001)

Parallelism: Biotherapeutics *vs* Biomarkers

- Biotherapeutics:
  - parallelism involves analysis of test samples after *in vivo* drug administration (incurred sample)
  - provides insight into whether or not biotransformation/ metabolism or serum-protein binding produces **potential interference**

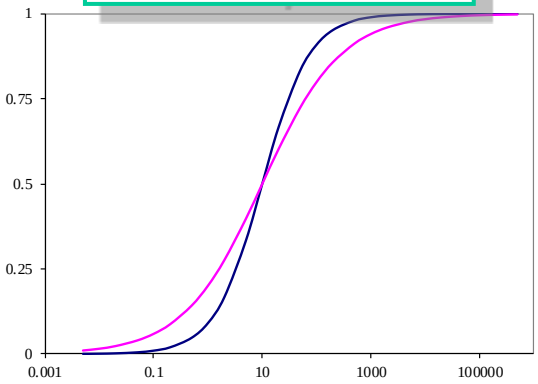
- Biomarkers:
  - **decrease/increase** in Biomarker concentration is only reliably in case parallelism between endogenous Biomarker and concentration-response curve (recombinant reference Biomarker) is demonstrated
  - Provides insight into whether or not a pharmacologic effect (i.e. protein biomarker) can be quantified

- **Detected by presence of nonlinearity after dilution**
- **Documents that concentration-response relationship of the analyte in samples from the study population is sufficiently similar to the analyte used in the calibration standards**

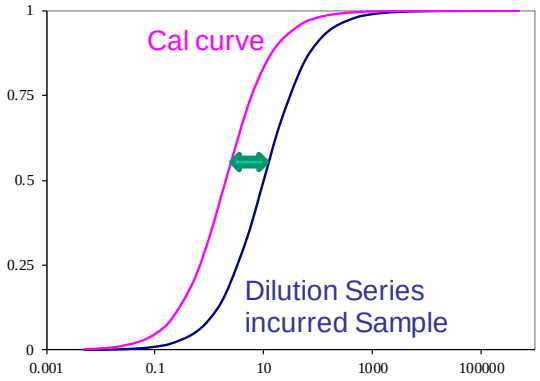
Parallelism *vs* Non-Parallelism

Might be OK for <u>Biomarker</u> quantification (relative accuracy)

Misinterpretation

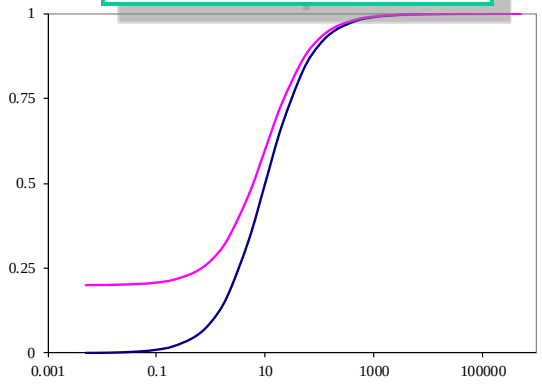


Change in responsiveness



Change in Antibody affinity

Misinterpretation

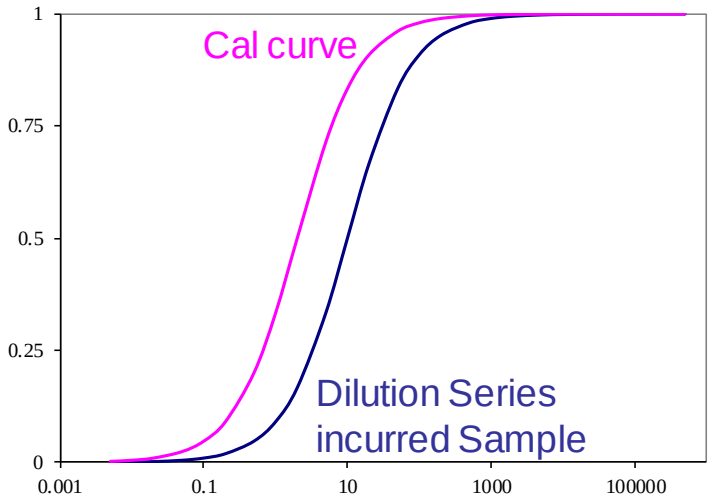


(A)specific binding of interfering substance

Might <u>NOT</u> be OK for <u>Biotherapeutic</u> quantification

Parallelism *vs* Non-Parallelism

Might be OK for <u>Biomarker</u> quantification (relative accuracy)



Might <u>NOT</u> be OK for <u>Biotherapeutic</u> quantification

➢ How to test?

- # incurred samples to assess?
- # dilutions into assay range?
  - Minimum span of dilution series (e.g. 10- or 50-fold)?
- Different approach for Biotherapeutics & Biomarkers?
- EMA specification of 30%CV precision sufficient?
- Statistical analysis recommended?

What is good/best practice for assessing Parallelism?

EMA guidance:

- serial dilution of high-concentration study sample to ≥ 3 concentrations in the assay range. *Specification: precision ≤ 30%CV*

DRAFT FDA guidance:

- Matrix effects: "Parallelism of diluted study samples should be evaluated with diluted standards to detect matrix effects
- Diagnostic kits: "If the analyte source (reference standards) in the kit differs from that of the subject samples (e.g., protein isoform variation), testing should evaluate differences in immunological activity with the kit reagents"

- **TT35 set out to provide DRAFT recommendation**
  - **Consolidate collected information**
  - **T35 Team might send out updated survey**
  - **Provide final recommendation**

T35 DRAFT Recommendation on Parallelism:

- **How to do it?**
- **What to do when it fails?**

Applicable to Quantitative Ligand Binding Assays

How to assess Parallelism?

- Select ≥ 6 study samples with high (endogenous) concentrations
- ≥ 3 dilutions into the assay range
- Precision of the diluted samples should be ≤ 30%CV
- No substantial trend in measured concentration as function of the applied dilution factor
- These acceptance criteria may be modified towards more stringent requirements if the deviation is supported by scientific rationale.

In case of Biotherapeutic quantification…..

1. Assess parallelism (≥ 6 incurred samples) as **early as possible** *in-study* (as soon as adequate samples are available).

2. PK data can be provided to clinical/development team as **draft** and is considered **valid** data in case **parallelism** can be **demonstrated**.

3. In case of **non-parallelism**, notify the clinical/development team that **data is invalid** and **cannot be reported/disclosed**.

4. Demonstration of **non-parallelism** requires further **investigation** of the analyte recovery as a function of the applied dilution.

5. **New assay design** may be required in case the phenomenon of non-parallelism cannot be explained and solved for the original assay.

6. **New assay reagents** might need to be qualified and **new assay** should be **validated** (including parallelism), using the samples from the current study.

7. Reanalysis of all study samples with new assay

In case of Biomarker quantification…..

1. Assess parallelism as early as possible ***pre-study*** with prototype assay, using appropriate validation samples (3-6 individual samples from healthy volunteers and/or samples reflective of, or derived from study population).

   Pre-study demonstration of **Non-parallelism** requires:
   - **re-evaluation** of the assay
   - decision to validate the assay for **quasi-quantitative purposes**

2. Establish **Go/No Go** to proceed with formal method validation for **quantitative** or **quasi-quantitative** purposes. The below recommendations apply to quantitative immunoassays.

3. Assess parallelism with additional samples (≥ 6 incurred samples) as **early as possible *in-study*** (as soon as adequate samples are available) if levels differ from that already tested *pre-study*, or if patient samples were not available *pre-study*.

4. Biomarker data can be provided to clinical/development team as **draft** data and is considered **valid data** in case **parallelism** can be demonstrated for a **quantitative assay**.

In case of Biomarker quantification…..
(continued)

5. In case of **non-parallelism**, notify the clinical/development team that **data is invalid** and **cannot be reported/disclosed** as originally planned.

6. Demonstration of **non-parallelism** requires further **investigation** of the analyte recovery as a function of the applied dilution.

7. **New assay design** may be required in case the phenomenon of non-parallelism cannot be explained and solved for the original assay.

8. New assay reagents/reference standard might need to be qualified and **new assay** should be **validated** (including Parallelism)**,** using the samples from the current study. Alternative: validation of a quasi-quantitative assay

9. Reanalysis of all study samples with new assay.

Concluding remarks

- Collect and Consolidate experiences from the community (currently limited case studies on non-parallelism)

- Update current recommendation – as required

The Team welcomes any input and suggestions!

Acknowledgement

- TT35
- All EBF members!