celerion

Translating Science to Medicine

Biosimilar Development: Lessons Learned From Early Clinical Studies

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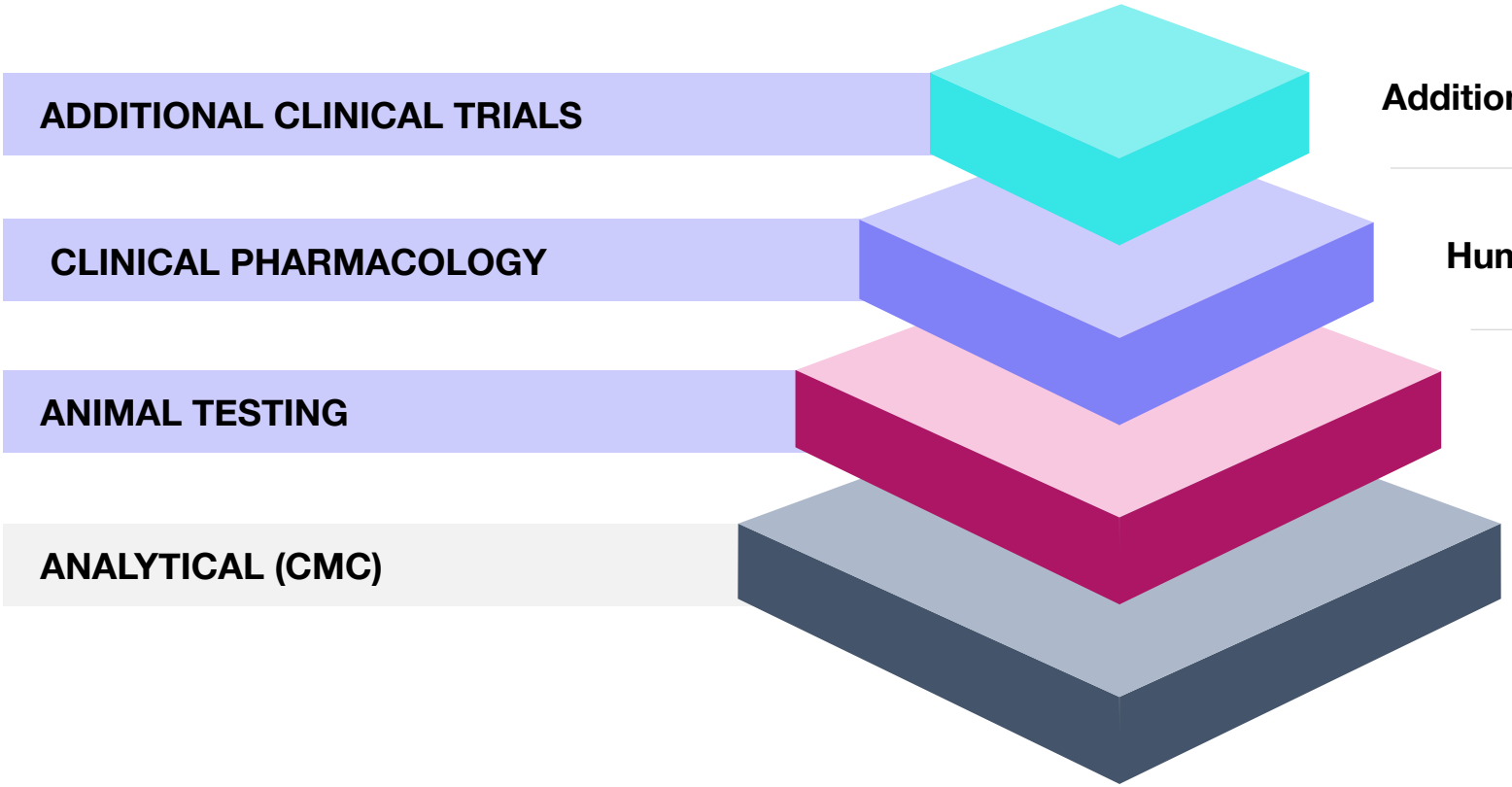
Totality-of-Evidence



Additional clinical data

Human PK/PD including immunogenicity

Compare all preclinical functions and confirm no enhancement of functions

Demonstrate analytical similarity (physicochemical and biological characterization)

Biosimilarity FDA: "….that the biological product is **highly similar** to the reference product notwithstanding minor differences in clinically inactive components" and that "there are **no clinically meaningful differences** between the biological product and reference product in terms of the safety, purity, and potency of the product…"

Section 351(k) Public Health Service Act (42 U.S.C. 262(k))

Main Challenge for bioanalytical studies supporting Biosimilars

When detecting concentrations of biosimilar and originator there are four main contributors to differences.

Subject to subject variability	**mostly taken care of by the study design (several periods, cross over)**
Lot-to-lot variability	**thorough characterization of the material used in the trial**
Chemical differences affecting the assay	**mostly by choosing a one- or two-assay-approach**
Precision of ligand-binding assays	**pushing the limit and automation**

One or two Assays?

Two Assay Approach	One Assay Approach
One assay for biosimilar One assay for reference drug	One assay can quantify **both** biosimilar and originator
Need to develop and validate **two assay**	Need to develop and validate **one assay**
Blinded analysis would require all samples to be run through both assays	Blinded study sample analysis possible

Reliability of the head-to-head comparison

J. Marini al, AAPS Journal (2014)

Two-step approach for one-assay setup

Development Phase

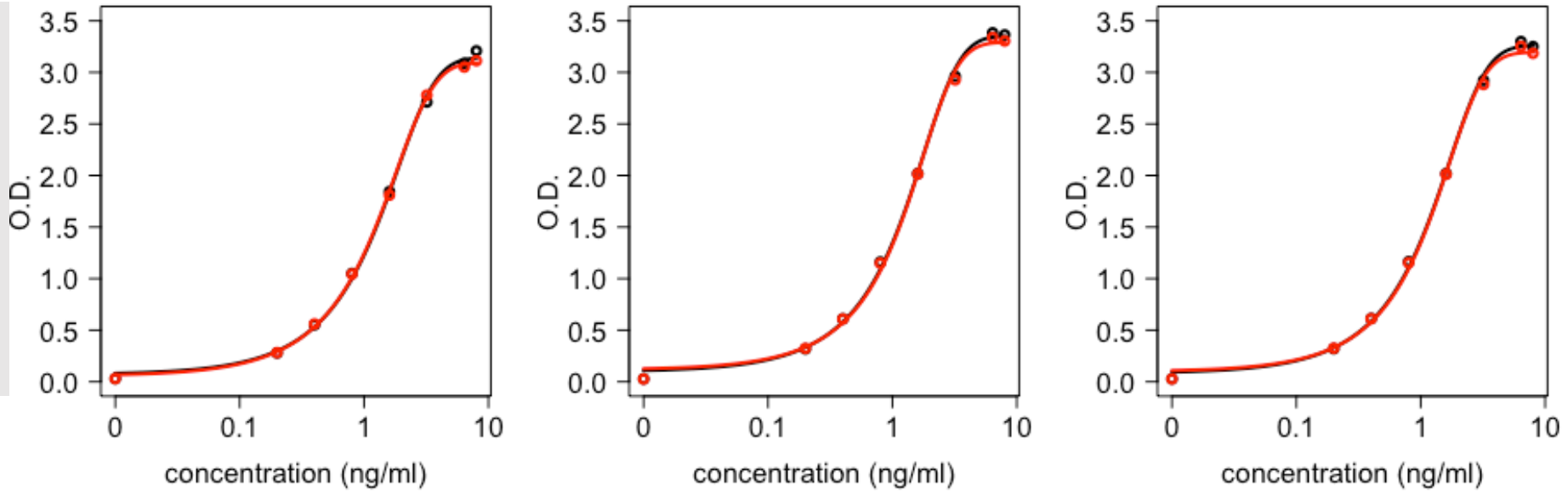
- establish bioanalytical similarity between calibration curves ≠ chemical similarity
- parallel or ideally superimposing

1. **Assay methodology**
2. **Critical reagents**
3. **Assay design (calibrators & quality controls)**
4. **Assay calibrators (biosimilar or originator)**

Validation Phase

- Confirmation of comparable reactivity of biosimilar and originator
- Systematic comparison of quality control results (intra/interbatch evaluation) of biosimilar and originator
- Direct comparison of both compounds with regards to other parameters, e.g. Selectivity, sensitivity, dilution linearity, stability

Establishing bioanalytical similarity

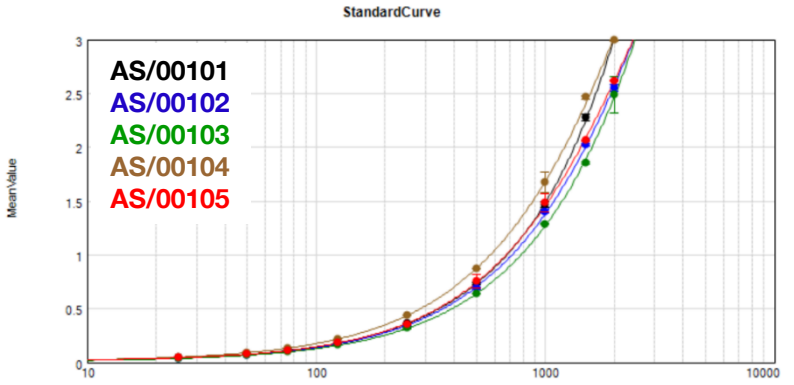


- Critical comparison of the standard curves needed
- Currently many proposed mathematical methods
- Overlaying curves as preferred approach (one assay approach)
- Perfectly parallel curves might indicate concentration differences (factor)
- Consistently non-parallel curves call for a two assay approach

Case Study 1: Standard curves comparison (CoA)

A

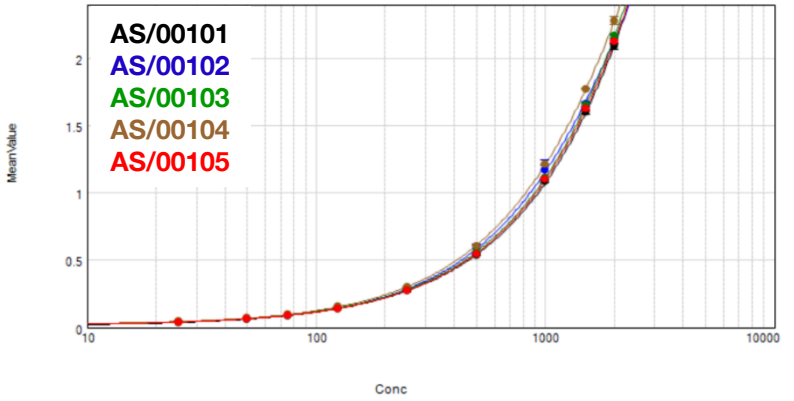
Standard curve preparation based on CoA concentrations:



	mean OD values (CoA)					
	AS/00101	AS/00102	AS/00103	AS/00104	AS/00105	% CV
STD 02	0.076	0.073	0.068	0.094	0.080	12.317
STD 03	0.112	0.106	0.098	0.132	0.110	11.483
STD 04	0.183	0.174	0.160	0.218	0.181	11.671
STD 05	0.363	0.346	0.318	0.439	0.358	12.314
STD 06	0.732	0.694	0.642	0.873	0.756	11.640
STD 07	1.468	1.403	1.288	1.675	1.487	9.647
STD 08	2.274	2.022	1.855	2.465	2.060	11.114
STD 09	N/AV	2.551	2.490	2.993	2.619	8.488
						11.084

B

Standard curve preparation based on concentrations measured by BCA protein assay:



	mean OD values (BCA)					
	AS/00101	AS/00102	AS/00103	AS/00104	AS/00105	% CV
STD 02	0.063	0.065	0.065	0.068	0.063	2.958
STD 03	0.089	0.090	0.091	0.099	0.090	4.183
STD 04	0.140	0.146	0.148	0.157	0.142	4.419
STD 05	0.272	0.283	0.282	0.304	0.277	4.344
STD 06	0.537	0.586	0.561	0.608	0.546	5.113
STD 07	1.089	1.175	1.110	1.210	1.100	4.643
STD 08	1.600	1.661	1.649	1.774	1.625	4.026
STD 09	2.089	2.145	2.170	2.281	2.122	3.398
						4.135

Case Study 2: Drug Substance vs Drug Product

A

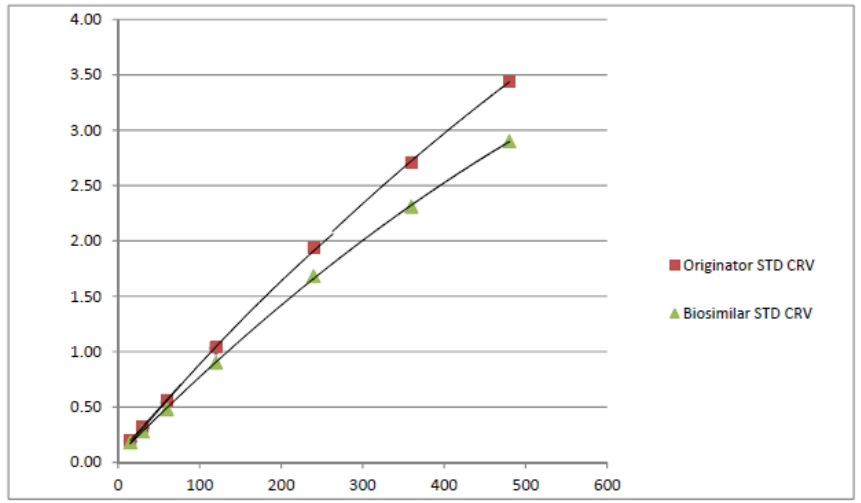
STD curve using provided "reference" material

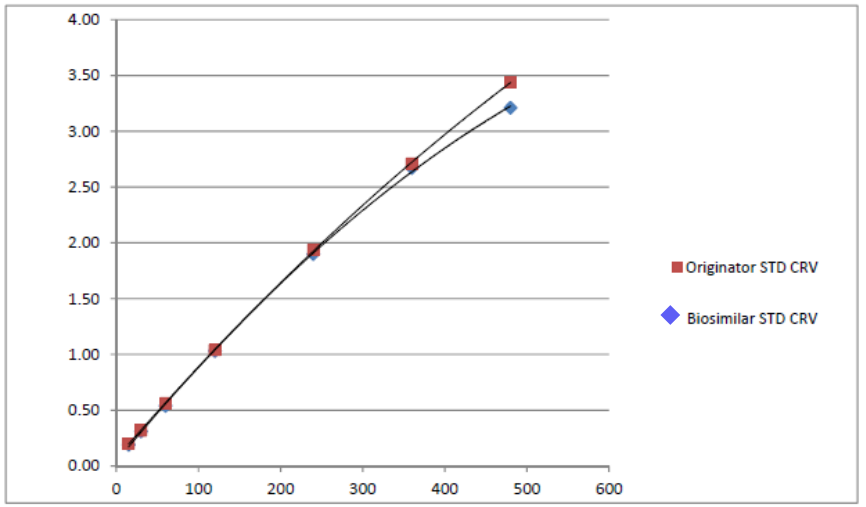
- Comparable concentrations according to CoA
- Drug product provided
- Packaging difference
  - Originator: glass
  - Biosimilar: plastic

B

STD curve comparison using Biosimilar reference substance:

- Successful biosimilar experiment
- Container material reviewed before start of clinical trial



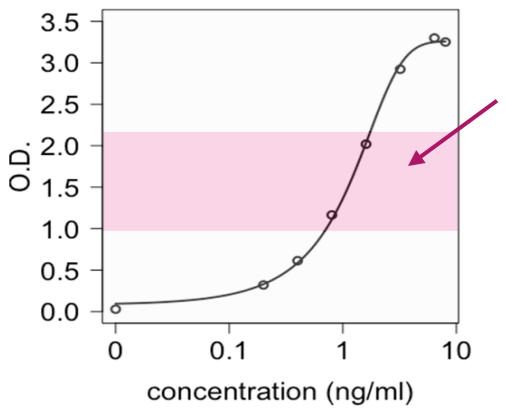


Proper certified drug substance is the key for successful bioanalytical PK methods

Case Study 3: Optimized Sample Analysis Set-up

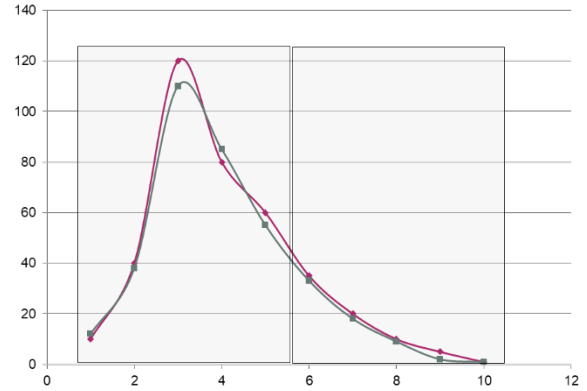
Need

- Sponsor needed a high statistical power in comparing originator and biosimilar
- Two stage study design with approx.
  - 170 subjects in stage 1
  - 100 subjects in stage 2



Approach

- Celerion scientists developed a robust assay and performed sample analysis with minimal inter-compound variability



Benefit

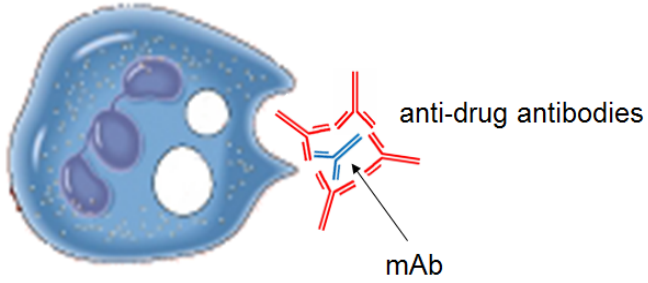
- Sponsor could meet all statistical endpoints and forgo the second stage
- Sponsor was able to avoid over 2 Million EUR in additional cost and 5 months of delay

Immunogenicity

- Immunogenicity occurs when the immune system recognises the administered test item as foreign and induces a humoral or cellular immune response.
- Multiple factors induce immunogenicity
  - e.g. Structural properties, Glycosylation, Aggregation, Impurities, Contaminants, changes in dosing schedule, administration routes, patient populations
- Can be a significant concern for biologics as it can affect both safety, efficacy and illicit life threatening events
- Head-to-head comparison with both, biosimilar and originator

FDA: "…Thus, establishing that there are no clinically meaningful differences of the immune response between a proposed product and the originator product.…"

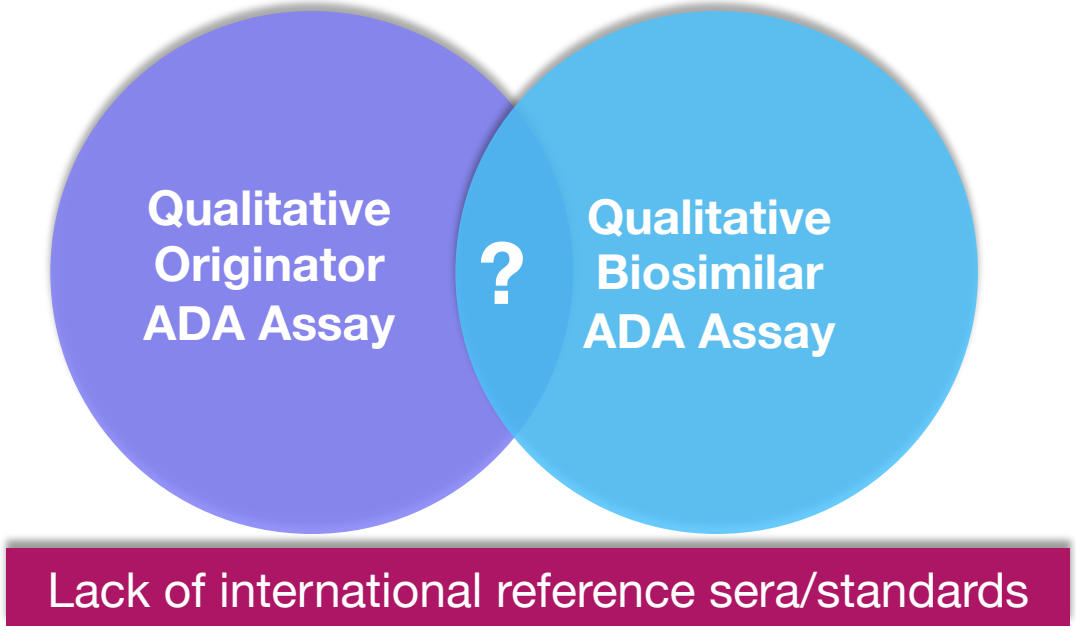
EMA: "…Analytical assays should be performed with both the originator and biosimilar molecules in parallel (in a blinded fashion)…"



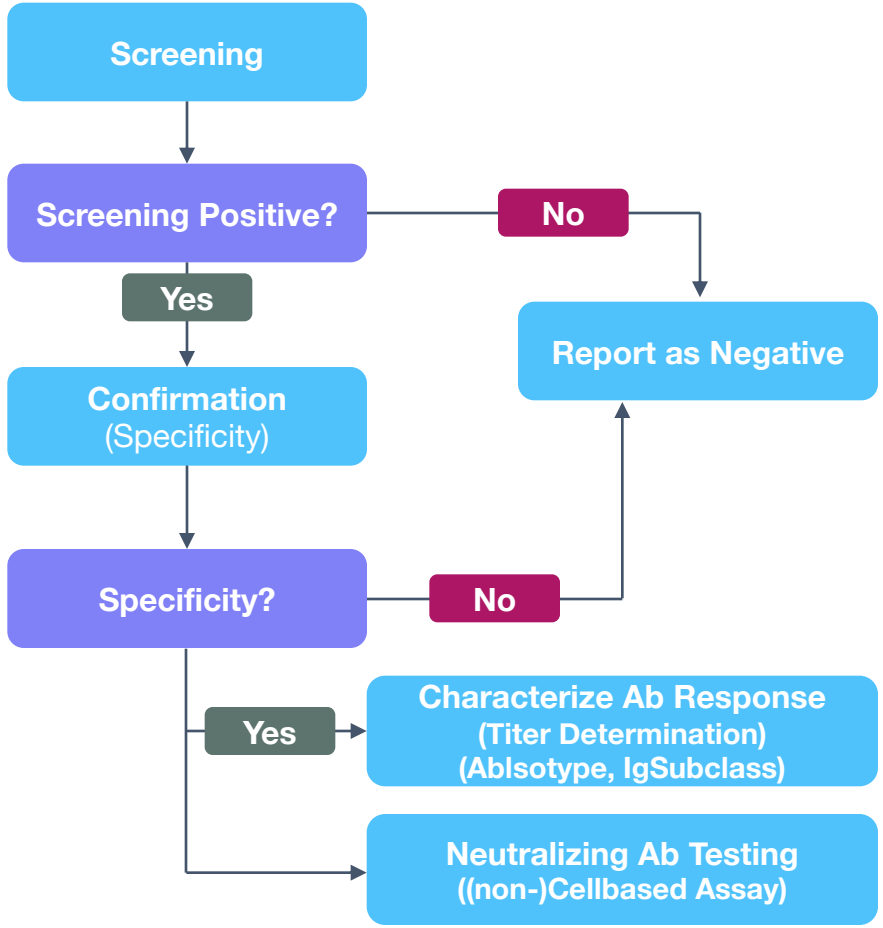
Comparative Immunogenicity (ADA) Testing

Evaluate potential difference between the biosimilar and the originator - not only to ensure safety and efficacy, but also allow for substitution and exchangeability

- The Biosimilar is **equally or less immunogenic** than the Originator
- ADA assays are inherently **qualitative in nature**



Lack of international reference sera/standards



F. Civoli al, AAPS Journal (2020)

Immunogenicity assessment

Platform & Methodology

- State-of-the-art technology should be used (= increase of sensitivity)

Positive Control

- Early on in the development control antibodies (positive controls) against the biosimilar and the originator should be generated in the same way
- Recommendation to use the biosimilar positive control, if only one is available

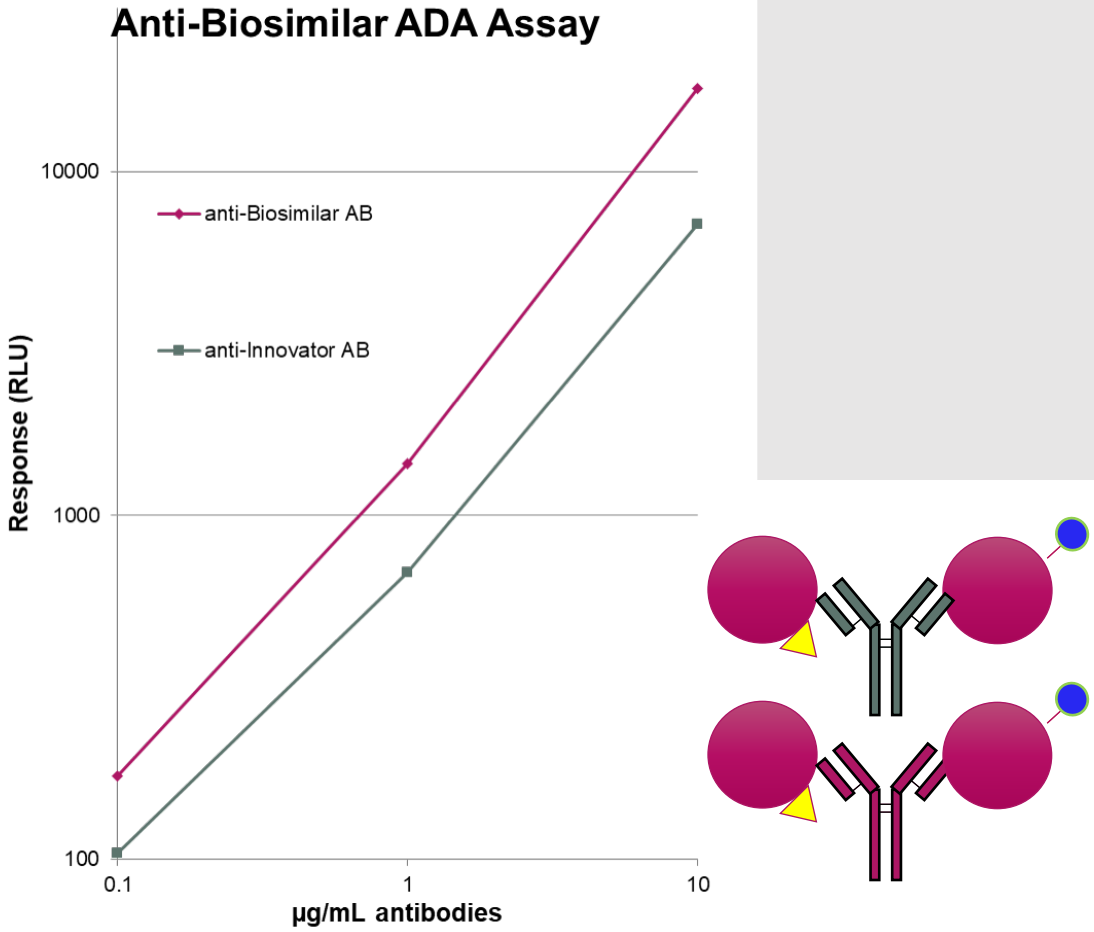
One assay approach - Antigenic Equivalance

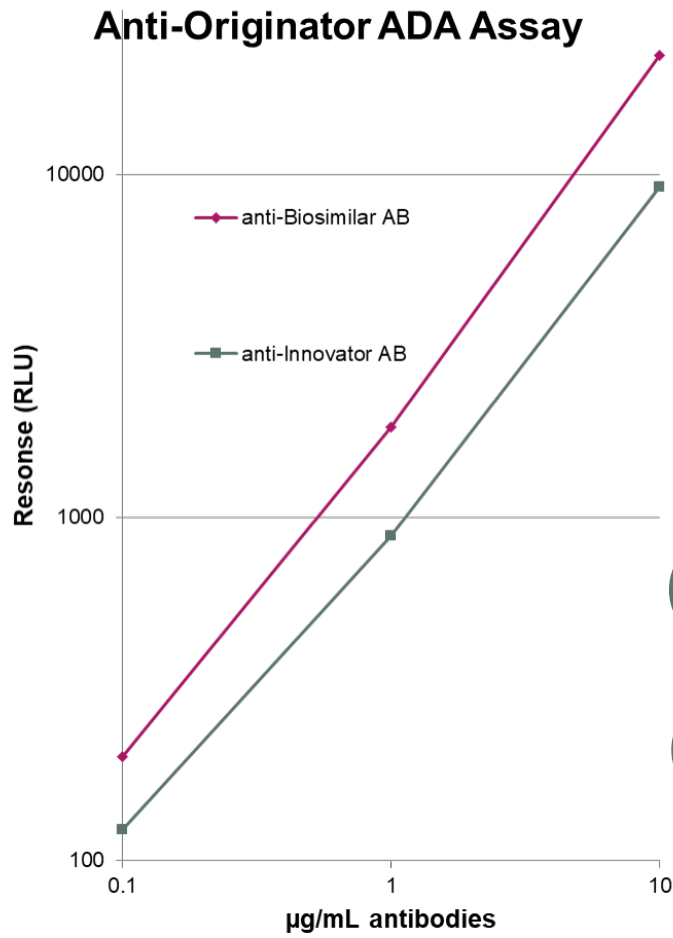
- Ensures that ADA against biosimilar are reliably detected
- All samples can be analyzed in one assay format
- ADA against the unique structure of the originator may not be detected

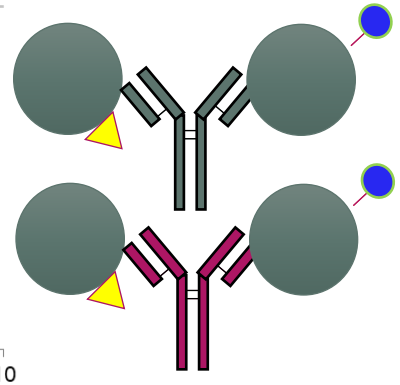
Sensitivity & Drug Tolerance

- Curves for biosimilar and Originator are comparable
- Sensitivity in the presence of interfering therapeutic drug product

ADA Assay Set-up

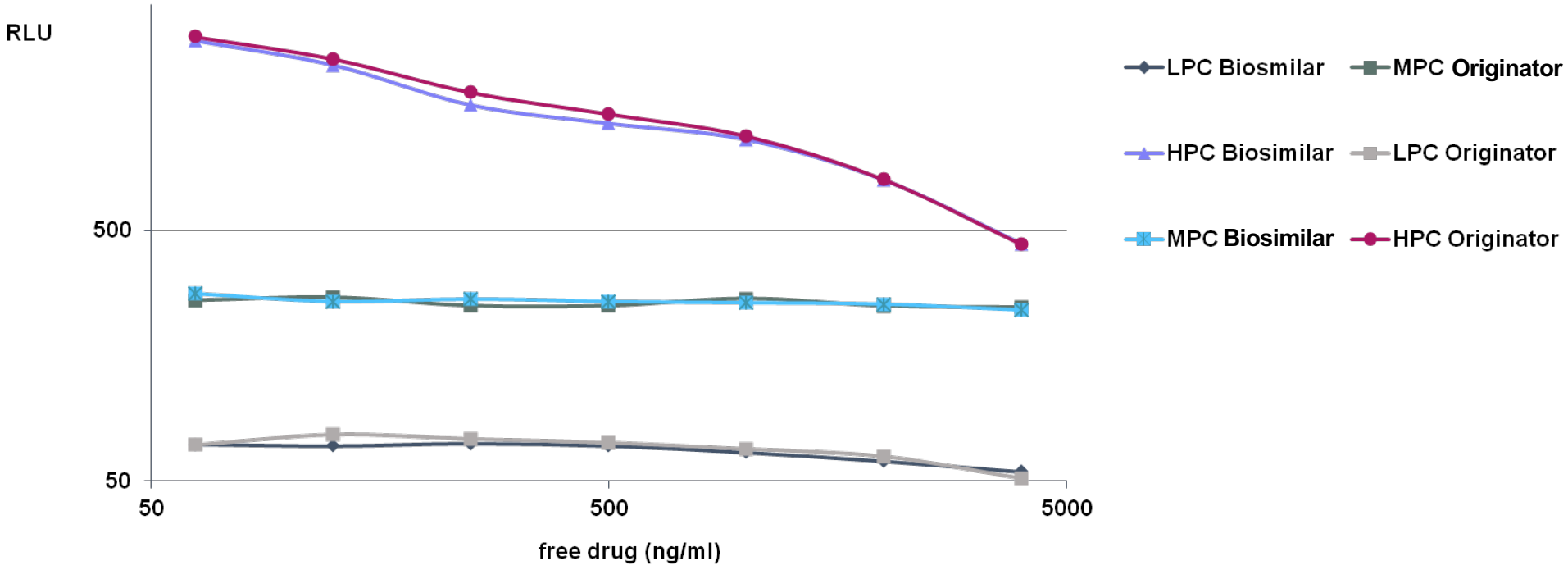






Drug competition curves (Antigenic Equivalence)

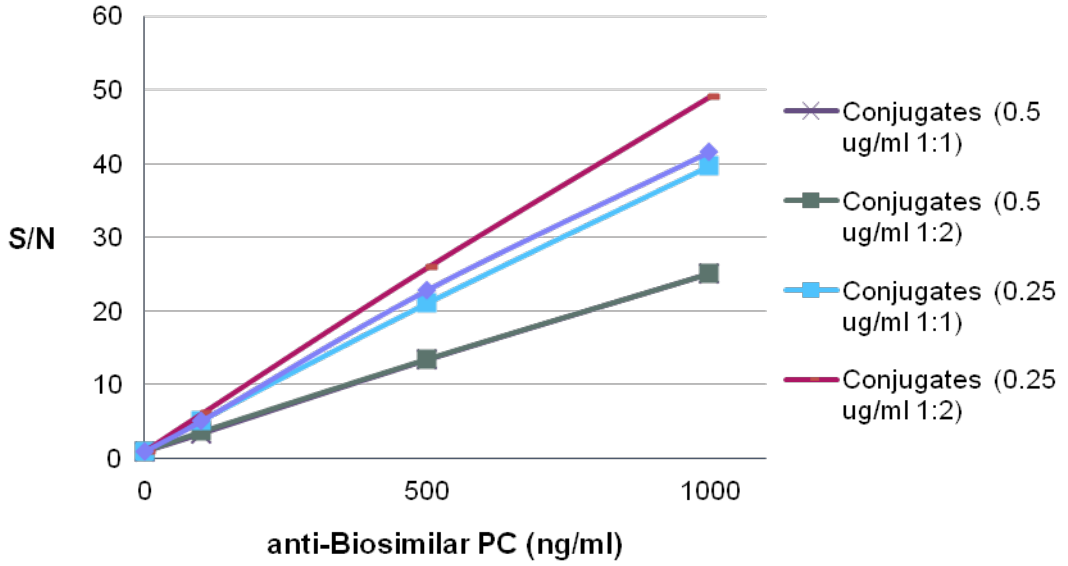
Biosimilarity Key Experiment: Free Drug Tolerance Biosimilar vs Originator



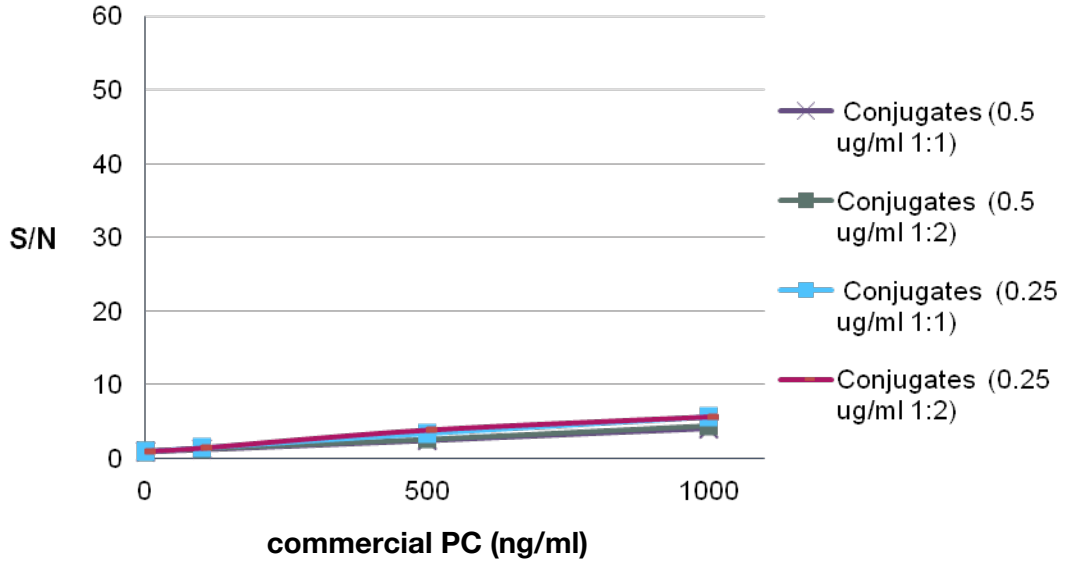
Biosimilar specific epitops are certainly recognized when the assay is developed using a biosimilar positive control

Case Study 4: Improved Sensitivity

A **Anti-Biosimilar positive control (specifically generated):**



B **Commercially available positive control (best out of 6 different ones):**



Improved sensitivity achieved using a specifically generated anti-biosimilar positive control

Neutralizing Antibody assessment

Assay Format

- Biologic's mechanism of Action (MoA)
- Evidence of desirable assay performance characteristic
- Risk of Immunogenicity

Assay Design

- Non-cellbased Assay
  - blocking of soluable target or cellular receptor
- Cellbased Assay
  - Suitable cell lines and proper assay endpoint

Assay Set-up

- One or multiple assay set-up
- Qualitative Screening set-up

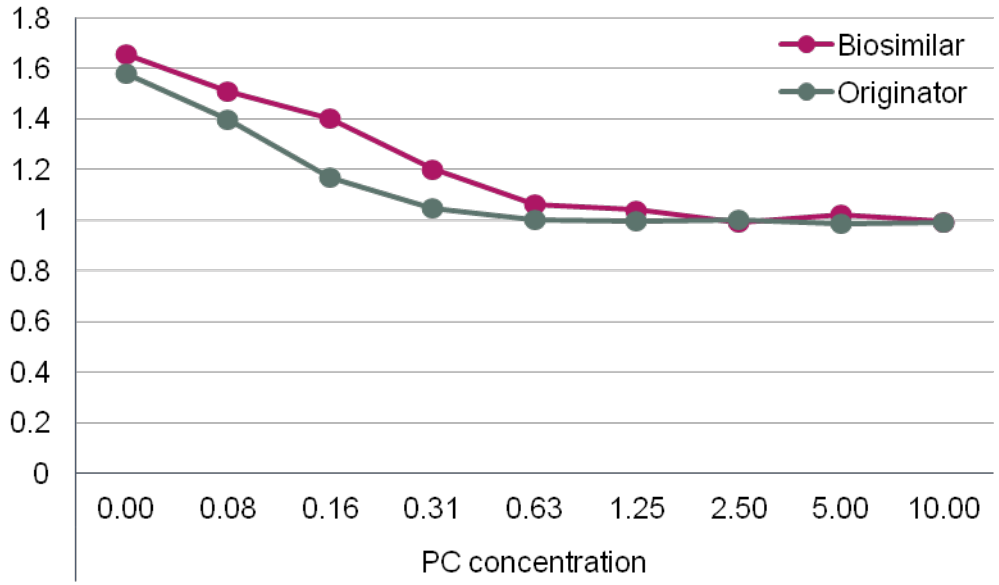
Positive NAb Controls

- One or more can be used, if one recommended to use the biosimilar
- Similar "sensitivity"

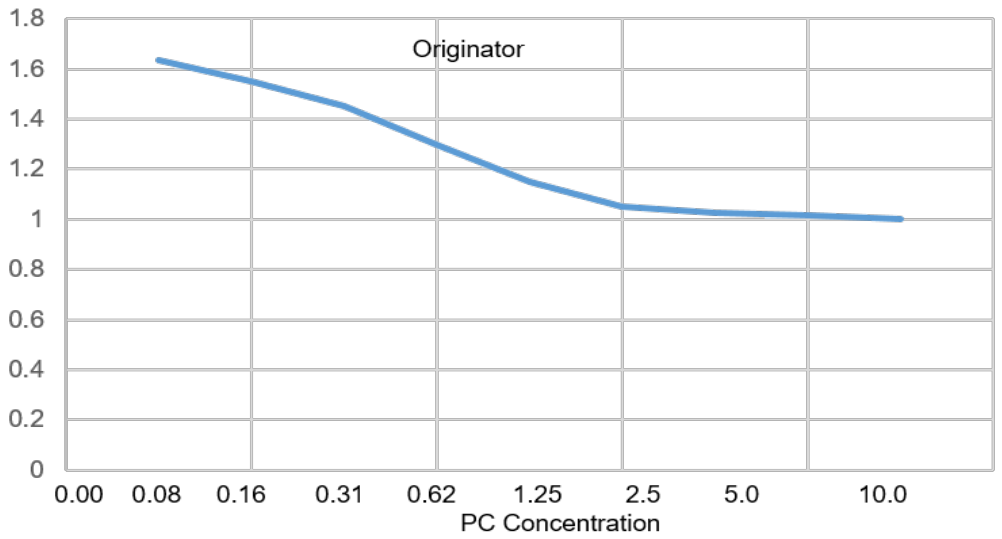
D. Gouty al, AAPS Journal (2018)

Case Study 5: Sensitivity of nAb assay

A **Sensitivity of a CBA nAB with anti-Biosimilar PC : 200ng/ml**



B **Sensitivity of a CBA nAB with a commercial anti-Originator PC : 600ng/ml**



Improved sensitivity achieved using a specifically generated anti-biosimilar positive control

Critical Learnings Based on our Experience

- Whenever possible apply the “One assay approach” using the biosimilar
- Ensure proper certification of all references
- Positive controls generated for the biosimilar can ensure all epitops on the biosimilar drug are picked up
- Positive controls generated for the biosimilar supports better sensitivity
- Bioanalytical assay set-up has a direct impact on the statistical endpoints of a trial