Analytical Bridging: How to Cross on the Wire Stretched Between Two Bioassay Methods?

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CASSS CMC Strategy forum Europe 2020

Virtual format

May 11-13, 2020

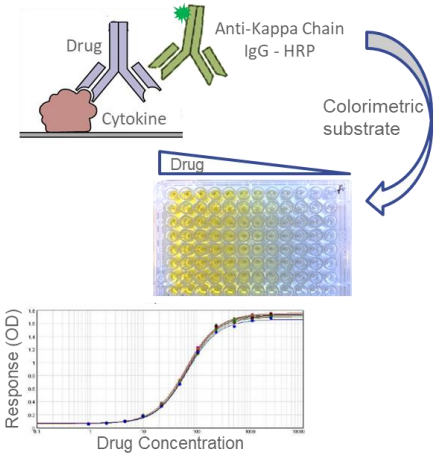
Inspired by patients.
Driven by science.

Before we start…

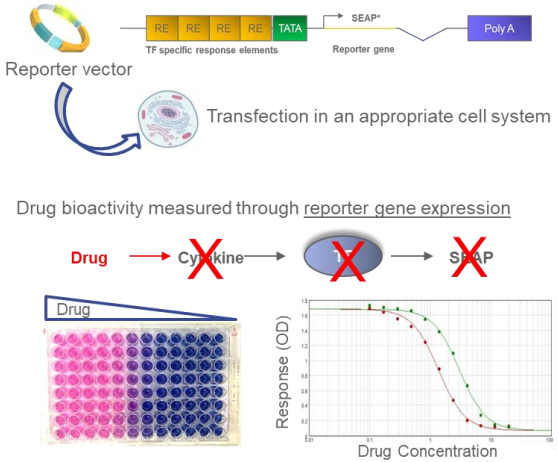
- Copy of the slides will be made available
- Feel free to contact me for any question, feedback or simply to debate: gael.debauve@ucb.com

ELISA First!

Early phase



Late phase



HOW???

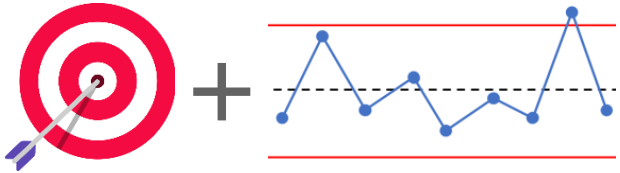
Design
Decision criteria
Methodology (stat?)

WHAT????

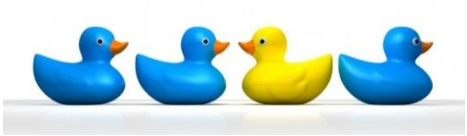
What to compare? Validation data / Routine results (sample set)

WHY!!!

Comparable results (no discontinuity between past and future data sets)



Abnormal samples detected with both methods



Differences are expected!*

Identify, characterize and evaluate the impact

* Not surprising when comparing 2 brothers or 2 methods that does not share the same principle/way to analyze data

How different are the 2 methods?

Parameter		Method A	Method B
Drug		IgG1	
Assay Principle		ELISA Binding of the drug to its target	Cell-based assay Impact of target neutralization on signaling cascade
Use		Early phase release and stability	Late phase release and stability
Routine layout	Assay format	1 bioassay = 3 plates	1 bioassay = 4 plates
	Replication strategy	Per plate: • dose-response = 11 concentration points • 2 independent standard preparation • 1 sample preparation	
Maximum variability of the reportable result based on routine layout*		8.5%	12.3%

* Calculated from a variance decomposition analysis performed on validation results

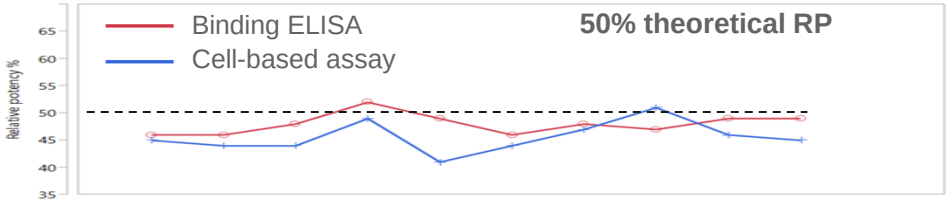
Head to head comparison of sample set

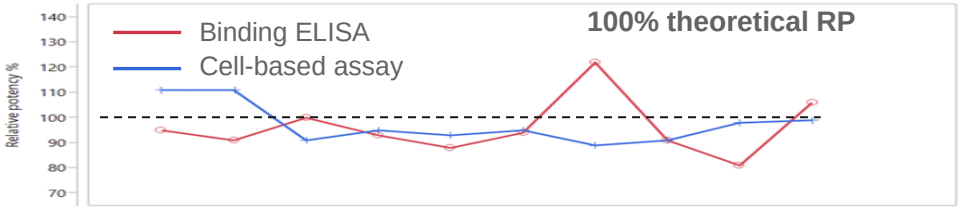
Stability samples	Degraded samples
n=30 (3X10 @ 50-100-200%RP)	n= 10
• DS and DP • Different manufacturing processes • Different storage conditions (from -70°C to 40°C) • Different timepoints (from 0 to 36 month)	Panel of various stress conditions

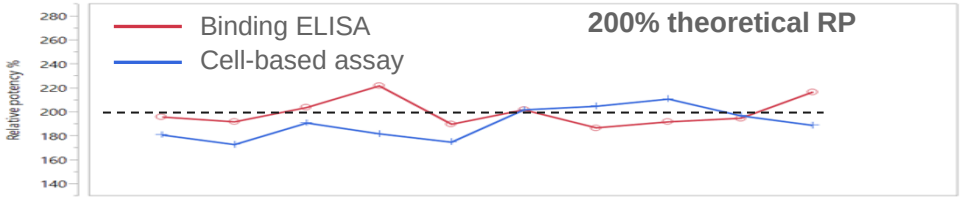
Stability samples

Looks comparable…

But how to confirm that statistically?

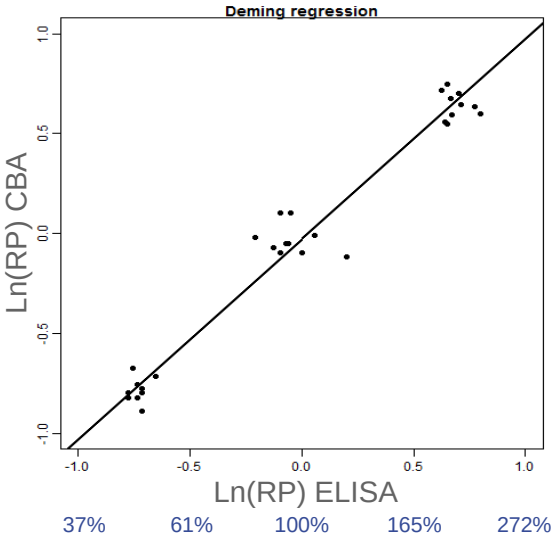






Statistical evaluation of stability sample data

The Deming regression



Statistical Parameter	Estimate	Std. Error	95% CI	Acceptance criteria	
Slope (b)	1	0.03	[0.95, 1.06]	Statistically not different to 1*	95% CI contains 1
Intercept (a)	-0.03	0.02	[-0.07, 0.01]	Statistically not different to 0*	95% CI contains 0

No significant difference between methods is detected + linear relationship

* 5% significance level

Outcome of the Deming regression

Pro:

Easy to implement and to draw conclusions
Provides the linear relationship* between the 2 methods

Limitation:

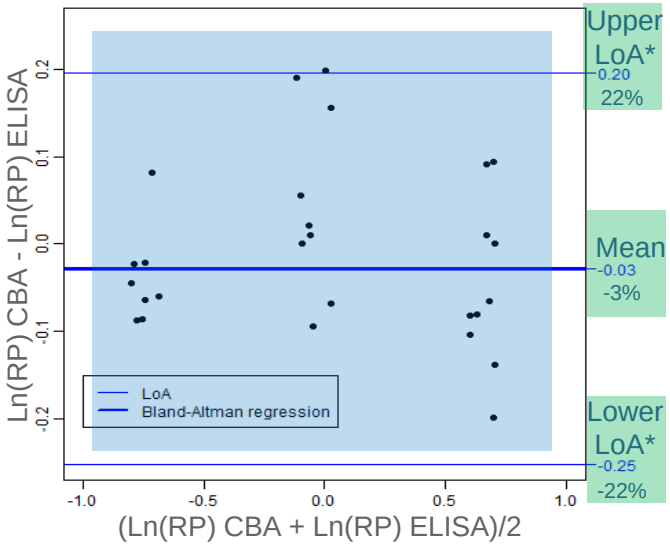
Regression could pass through highly variable results (and conversely)

Use a second approach to better visualize the random error/systematic bias and confirm that results are comparable

* direct equation between LnRP A and LnRP B

Statistical evaluation of stability sample data

The Bland-Altman difference plot



- Accepted bias = ±22%
- On average, CBA underestimates results by 3% compared to ELISA

- Values visually randomly and independently distributed around the difference mean value
- No trend (variance of the difference is constant over the tested range)
- No outlier detected

Results from both methods are comparable!

* Limit of Agreement (LoA) calculated as mean ± 2SD

Outcome of the statistical evaluation on stability samples

Deming regression and Bland-Altman outputs are aligned

Results generated by both methods on stability samples can be considered as comparable!

… what about the sensitivity to pick up product degradations?

Head to Head comparison of forced degradation samples

Stress type	Condition
Temperature	50°C/14days
pH	pH3.0/14 days
	pH10.0/14 days
Deamidation	1% Ammonium Bicarbonate pH8.1 @40°C/14days
Light	1000 Klux hours
	2500 Klux hours
	5000 Klux hours
Glycation	0.5M Glucose @37°C/91h
	0.5M Glucose @47°C/91h
Oxidation	1% H_2O_2/14 days
	15%AAPH* @47°C/14 days

* 2,2′-azobis (2-amidinopropane) dihydrochloride = a free radicals generator used to study the effects of oxidative stress

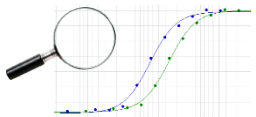
Head to Head comparison of forced degradation samples

Stress type	Condition	%RP ELISA	%RP CBA
Temperature	50°C/14days	Similarity SST fail	81
pH	pH3.0/14 days	Similarity SST fail	69
	pH10.0/14 days	Similarity SST fail	92
Deamidation	1% Ammonium Bicarbonate pH8.1 @40°C/14days	90	101
Light	1000 Klux hours	96	88
	2500 Klux hours	100	95
	5000 Klux hours	86	79
Glycation	0.5M Glucose @37°C/91h	70	89
	0.5M Glucose @47°C/91h	100	89
Oxidation	1% H_2O_2/14 days	103	86
	15%AAPH @47°C/14 days	Similarity SST fail	Below LLOQ

No impact on bioactivity detected with any of the 2 methods

Impact on bioactivity detected with 1 of the 2 methods

Impact on bioactivity detected with the 2 methods



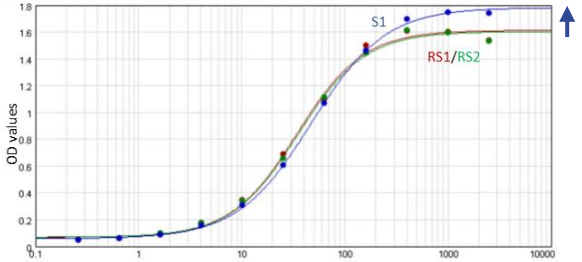
ELISA and CBA differently impacted

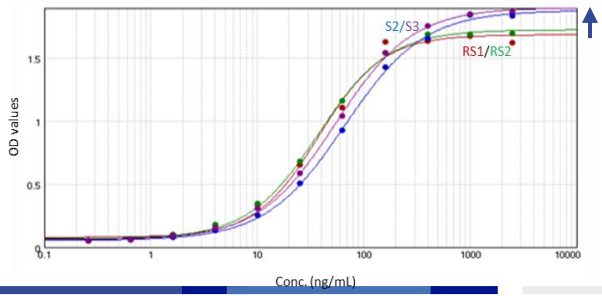
Stress type	Condition	%RP ELISA	%RP CBA
Temperature	50°C/14days	Similarity SST fail	81
pH	pH3.0/14 days	Similarity SST fail	69
	pH10.0/14 days	Similarity SST fail	92

Temperature stress

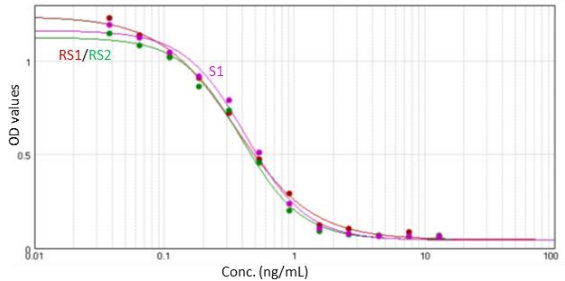
pH 3 and 10

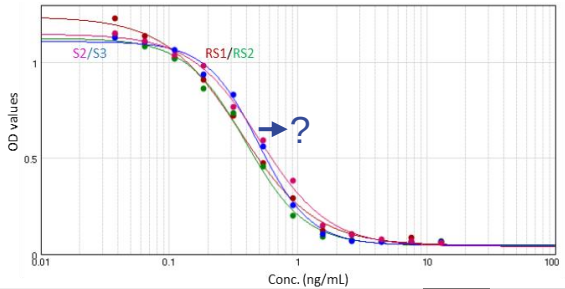
ELISA





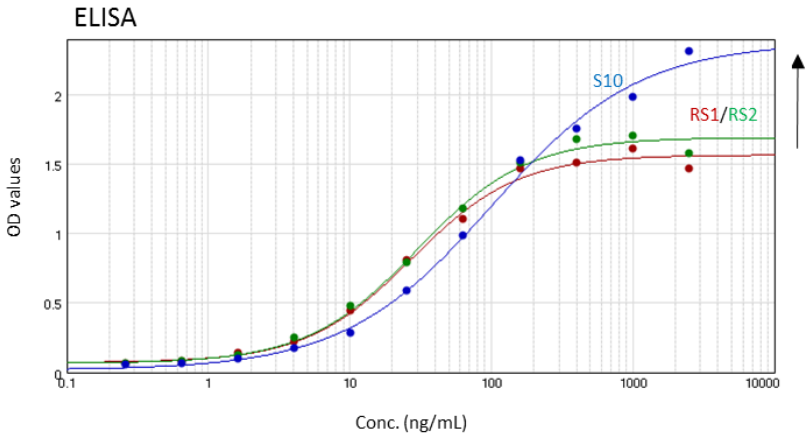
CBA





Impact clearer with AAPH oxidation

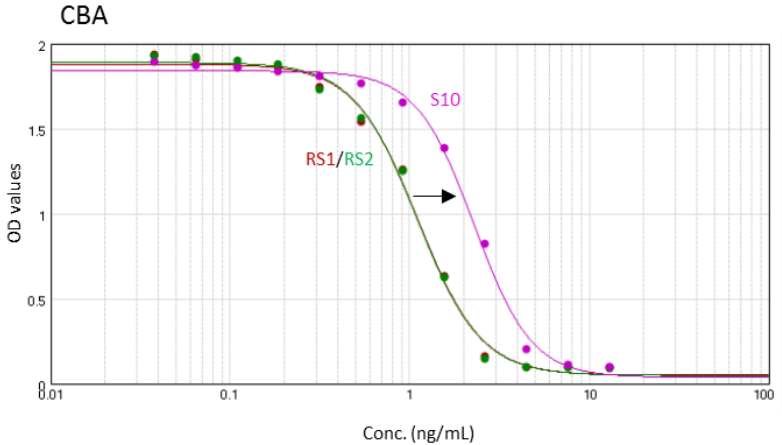
Stress type	Condition	%RP ELISA	%RP CBA
Oxidation	15%AAPH @47°C/14 days	Similarity SST fail	Below LLOQ



Upper asymptote clearly impacted

➔ **Impact on similarity** (SST fail)

➔ RP is meaningless



Curve parameters (asymptotes and slope not impacted)

➔ **No Impact on similarity**

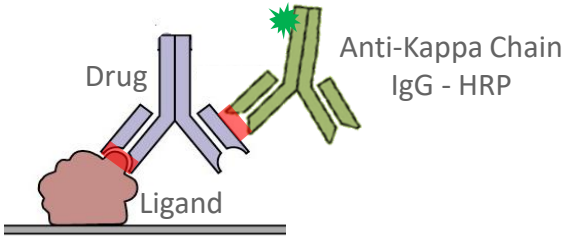
➔ RP is calculated (< 50% (LLoQ))

Bioactivity is clearly affected in both methods but not in the same way (similarity vs drop in RP)…

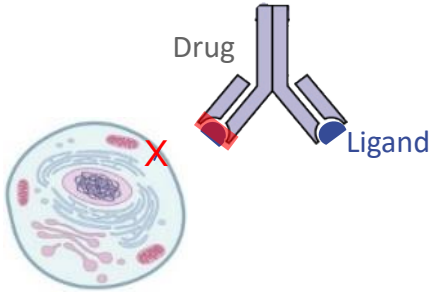
What could explain that difference?

Focus on bioassay format driven differences

… and particularly on the IgG regions involved in the signal



CDR + Kappa chain

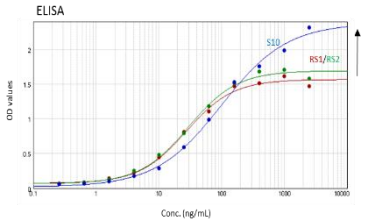


CDR only

- CBA format is closest to what happens *in vivo*
- More chance to have dose-response relationship impacted by degradation in ELISA as 2 “Achilles’ heels” instead of 1 in CBA

BUT…

- Impact of degradations on drug kappa chain not clearly demonstrated by higher order structure analyses
- kappa chain does not explain the signal increase observed in ELISA



Widen the perspective

Can we connect bioassay data with the other forced degradation results?

Support from forced degradation study

Degradation pathway was extensively characterized using a wide variety of analytical techniques (iCE, SPR, AUC, DSC, CD, intact mass, disulfide mapping …)

No common denominator for temperature, pH and AAPH stress conditions …

Stress type	Condition	%RP ELISA	%RP CBA
Temperature	50°C/14days	Similarity SST fail	81
pH	pH3.0/14 days	Similarity SST fail	69
	pH10.0/14 days	Similarity SST fail	92
Deamidation	1% Ammonium Bicarbonate pH8.1 @40°C/14days	90	101
Light	1000 Klux hours	96	88
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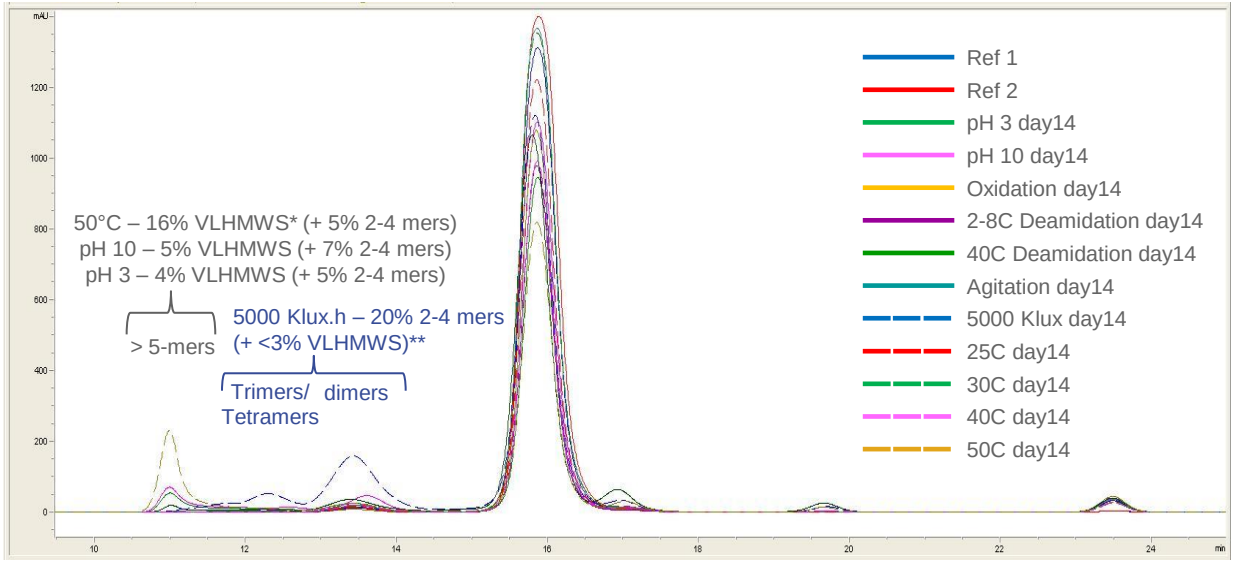
Glycation	0.5M Glucose @37°C/91h	70	89
	0.5M Glucose @47°C/91h	100	89
Oxidation	1% H_2O_2/14 days	103	86
	15%AAPH @47°C/14 days	Similarity SST fail	Below LLOQ

With the exception of SE-HPLC results

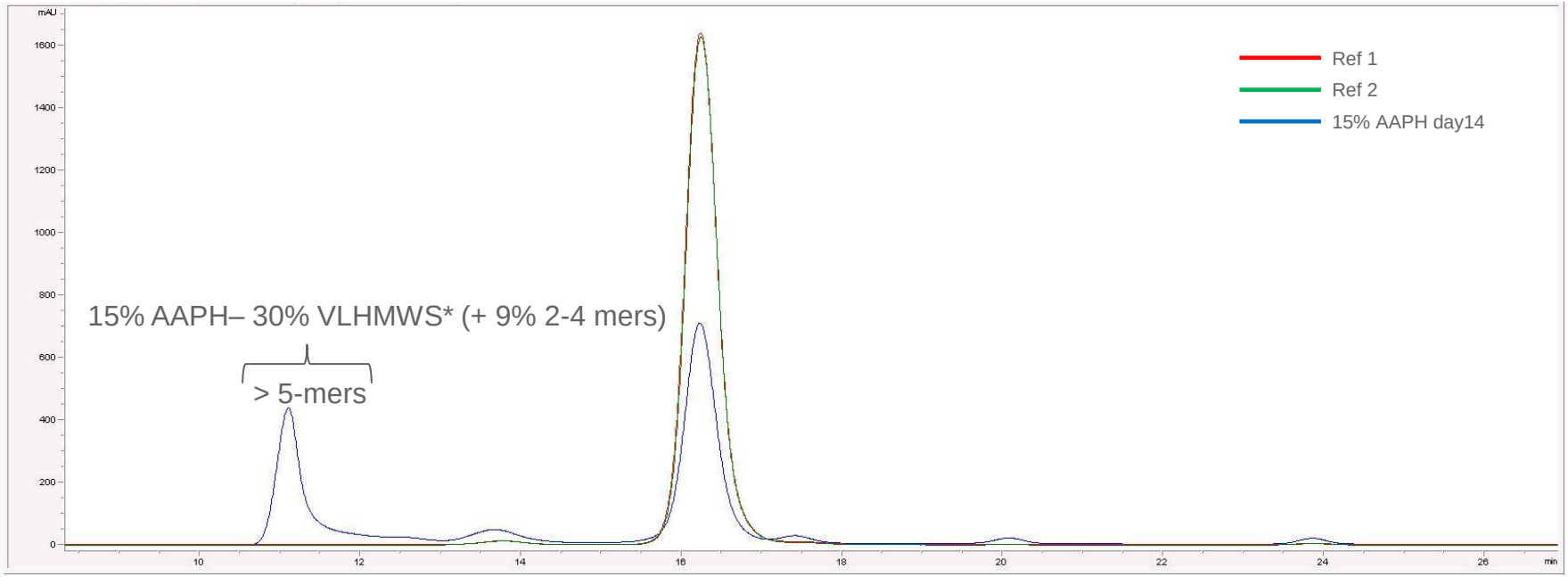
very large complexes (> 5-mers) observed with temperature and pH stress only

What about AAPH???



* VLHWMS = Very Large High Molecular Weight Species
** 1000 Klux.h = 6% 2-4 mers (VLHMWS not detected) , 2500 Klux.h= 11% 2-4 mers (VLHMWS not detected), data not shown

SEC-HPLC 15% AAPH



Very large complexes more abundant than in the other stress condition

* VLHWMS = Very Large High Molecular Weight Species (> 5-mers)

Holistic view of bioassay data

Stress type	Condition	%RP ELISA	%RP CBA	Very large HMWS
Temperature	50°C/14days	Similarity SST fail	81	Yes (> 3%)
pH	pH3.0/14 days	Similarity SST fail	69	Yes (> 3%)
	pH10.0/14 days	Similarity SST fail	92	Yes (> 3%)
Deamidation	1% Ammonium Bicarbonate pH8.1 @40°C/14days	90	101	No (<3%)
Light	1000 Klux hours	96	88	No (<3%)
	2500 Klux hours	100	95	No (<3%)
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Glycation	0.5M Glucose @37°C/91h	70	89	No (<3%)
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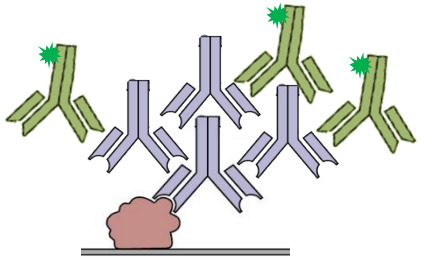
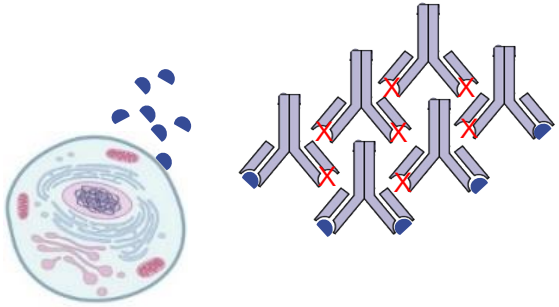
Oxidation	1% H_2O_2/14 days	103	86	No (<3%)
	15%AAPH @47°C/14 days	Similarity SST fail	Below LLOQ	Yes +++

What is the link between very large complexes and impact on bioactivity?

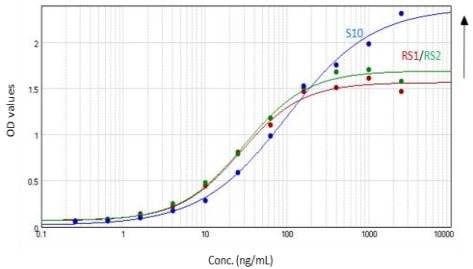
Can very large complexes explain ELISA/CBA differences?

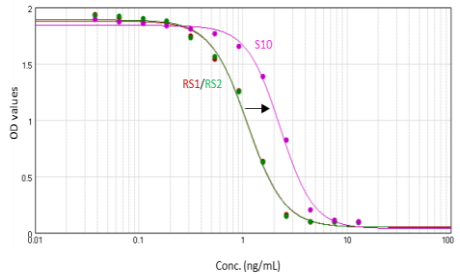


“Branching effect” mediated by secondary Ab leading to **signal amplification**



Competition between IgG/IgG and IgG/ligand interaction ➔ more free ligand leading to **delay in signal inhibition**

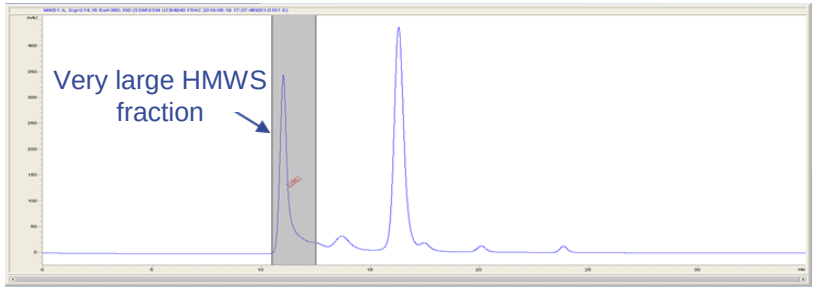




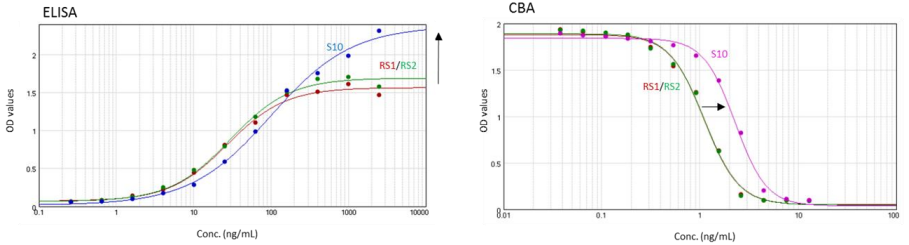
Link between very large complexes and assay format supports the increase in max signal observed in ELISA and the decrease in RP observed in CBA!

Can very large complexes explain ELISA/CBA differences?

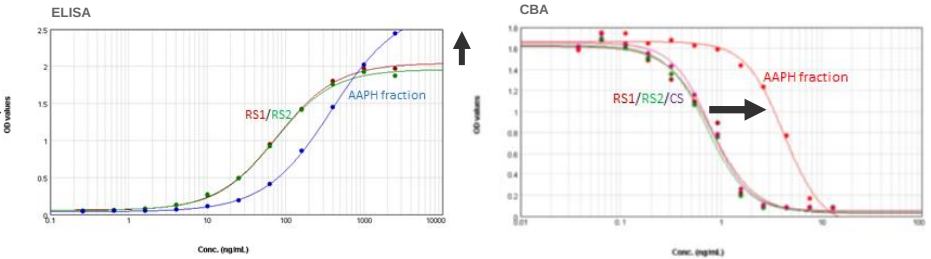
… confirmatory experiment on purified very large HMWS fraction from 15% AAPH degraded sample



Unpurified (original)



Purified very large HMWS



Curve shift even more pronounced with purified very large HWMS fraction
➔ Strengthen the link between very large HMWS and impact on bioactivity

Bridging general conclusion

- Results generated by both methods on stability samples can be considered as comparable
- Whilst forced degradation results are not identical, differences have been evaluated and characterized
- Very large complexes could potentially impact the biological activity
- ELISA looks more sensitive… but seems related to the way very large complexes impacts the dose-response relationship in the ELISA format (similarity)
- Risk of very large complexes impacting bioactivity is mitigated by SE-HPLC (more sensitive and part of the release/stability package (↑ detectability))
- Cell-based assay is more representative of the drug mechanism of action

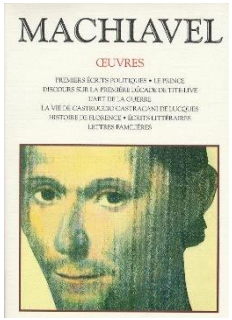
➔ In this case study:
CBA ≥ ELISA

Take home message

StatisK Ycians

Think about the objective(s), raise the good question(s), apply the right statistical tool(s)

Put an appropriate level of energy in identifying the design of assay bridging and identifying the potential pitfalls



Keep your "*stability samples*" close but your "*forced degradation samples*" closer

- ✓ Time consuming
- ✓ Holistic view - put bioassay in perspective with other analytical data
- ✓ Differences are not necessarily unacceptable, as far as they are characterized and impact is evaluated

The Bioassay Dev "A-Team"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Lauriane Mongodin


Caroline Kummert


Fouad Eddahri


Morgane Gesquière


Bastien Cornet


Patricia Van Brackel


Christophe Marchi

The Non Clinical Biostatistician Charlie's Angels


Bianca Teodorescu


Hanitsoa Rakotosalama


Dimitris Gayraud

The Characterization Crew


John O'Hara


Andreia Marques


Katharina Lang

The Phys/Chem Squad


Carl Jone


Annick Gervais


Michel Degueldre


Sandrine Van Leugenhaeghe

Questions?

Thanks!