Case studies of Issues with Stability of Antibody Reagents

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Definition of assay reagents

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Ligand Binding Assay **Reagents** – not Drugs (Analytes, Reference standard)

Distinction between:

Critical reagents	-	Non-critical reagents
Principal/Key reagents	–	Other reagents
Assay specific reagents	-	Auxiliary reagents

„Assay (specific) reagents are those that are responsible for the sensitivity and selectivity of the assay, e.g. monoclonal or polyclonal antibodies, receptors, target ligands, peptides that have the potential to recognize specific sequences or structural features that are unique to the analyte" *(B. Rup and D. O'Hara ,The AAPS Journal 2007; 9 (2) Article 16)*

Guidelines

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EMEA Guideline on bioanalytical method validation 2011:

„Critical reagents.....their **quality must be assured**. ...when changing reagent batches ... the **analytical performance must be verified**.... Conditions guaranteeing the **maintenance of the stability**...should be documented....“

FDA Guidance 2001

„Key reagents.....should be characterized appropriately and stored under defined conditions. ....Assay reoptimization or validation may be important when there are changes in key reagents...“

Viswanathan C.T: AAPS Journal 9(1) 2007, E30-E42

„...documentation should be made of the conditions under which the principal reagents maintain sufficient stability.... **Some of these (stability) data will need to be generated by the sponsor in the case of proprietary reagents.....**“

How to investigate reagent stability?

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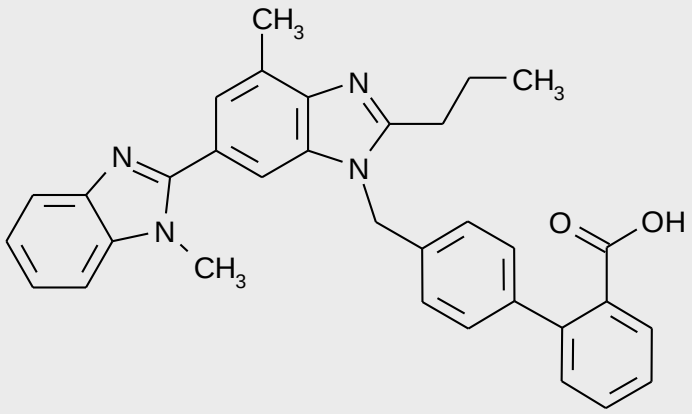
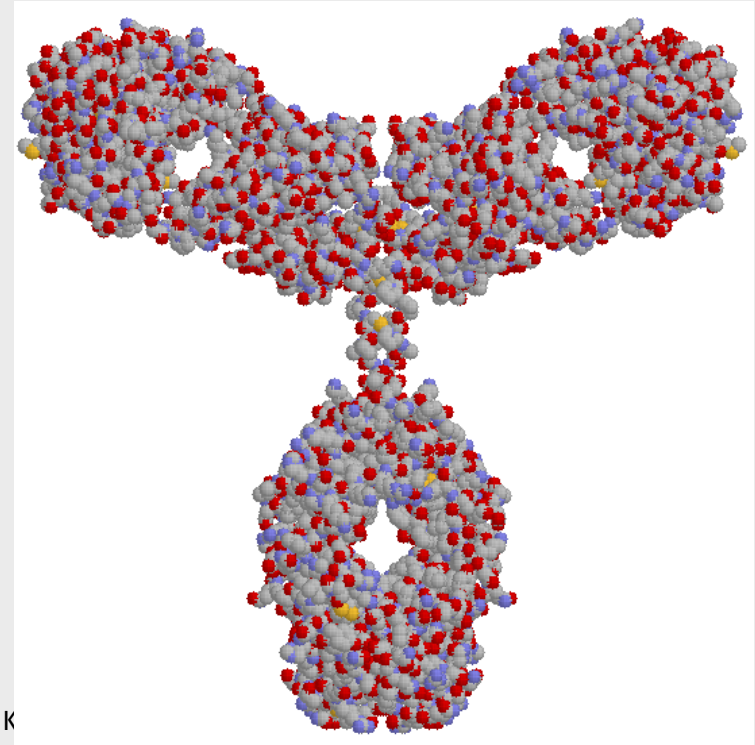
1. Approach:
   Comprehensive characterization of structure and reactivity of reagents prior and after stress and storage conditions (physico-chemical characterization + binding assays)

2. Appoach:
   Revalidation of method the reagents are intended for, after certain times

3. Approach:
   Do nothing! Trust on the batch acceptance criteria as proof for reagent stability

1. Approach: Stability investigation by repeated full characterization of molecule - Would this be feasible?





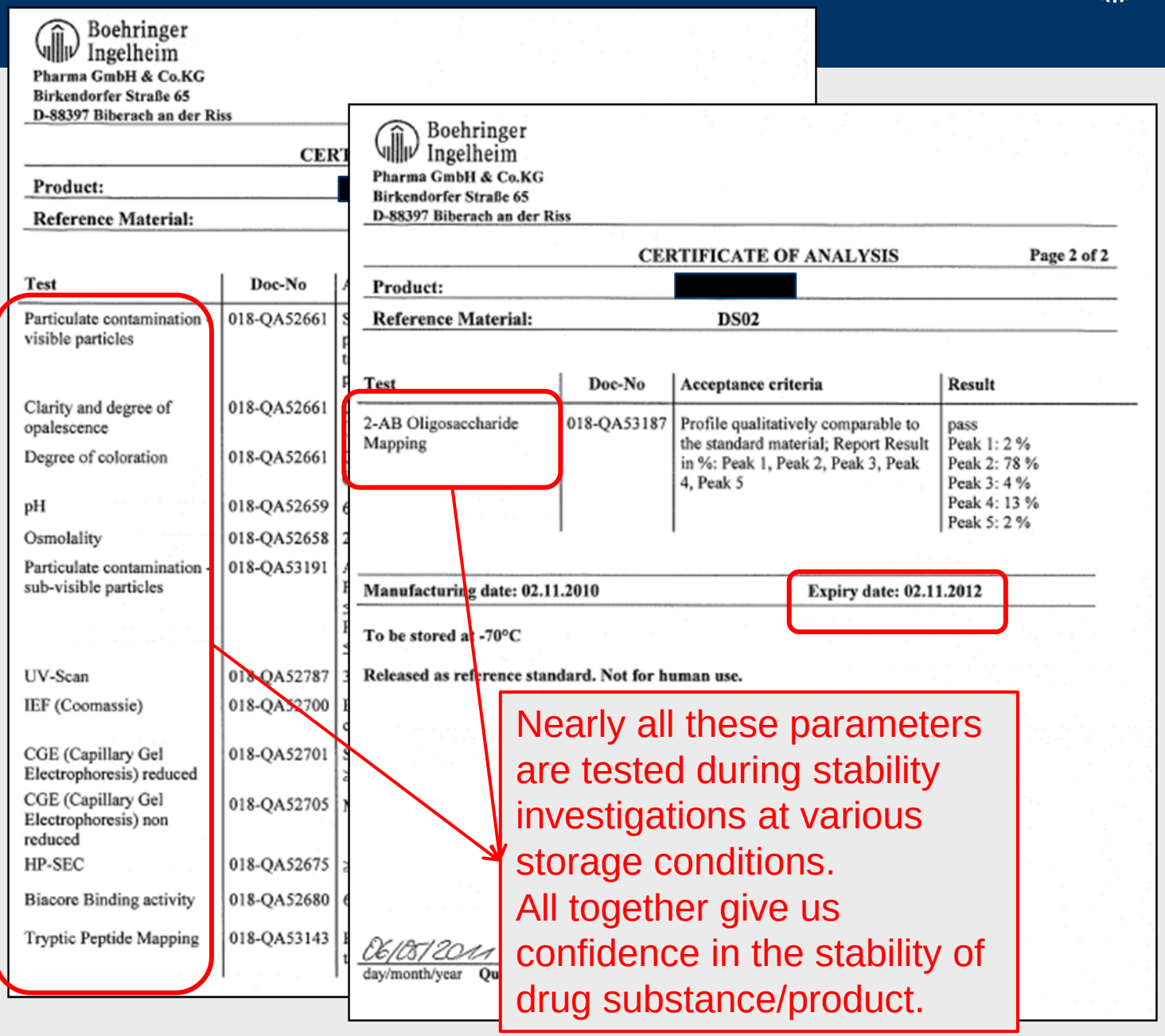
Small chemical molecules:
Stability and Purity tested with physico-chemical analytical methods, e.g.:

- Elemental analysis
- NMR
- MS
- HPLC

-> **sufficient proof**

How to test identity and assess stability of an antibody and other assay specific reagents?

1. Approach: GMP effort for therapeutic proteins



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CERT

Product:

Reference Material:

Test	Doc-No
Particulate contamination - visible particles	018-QA52661
Clarity and degree of opalescence	018-QA52661
Degree of coloration	018-QA52661
pH	018-QA52659
Osmolality	018-QA52658
Particulate contamination - sub-visible particles	018-QA53191
UV-Scan	018-QA52787
IEF (Coomassie)	018-QA52700
CGE (Capillary Gel Electrophoresis) reduced	018-QA52701
CGE (Capillary Gel Electrophoresis) non reduced	018-QA52705
HP-SEC	018-QA52675
Biacore Binding activity	018-QA52680
Tryptic Peptide Mapping	018-QA53143

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CERTIFICATE OF ANALYSIS Page 2 of 2

Product:

Reference Material: DS02

Test	Doc-No	Acceptance criteria	Result
2-AB Oligosaccharide Mapping	018-QA53187	Profile qualitatively comparable to the standard material; Report Result in %: Peak 1, Peak 2, Peak 3, Peak 4, Peak 5	pass Peak 1: 2 % Peak 2: 78 % Peak 3: 4 % Peak 4: 13 % Peak 5: 2 %

Manufacturing date: 02.11.2010

Expiry date: 02.11.2012

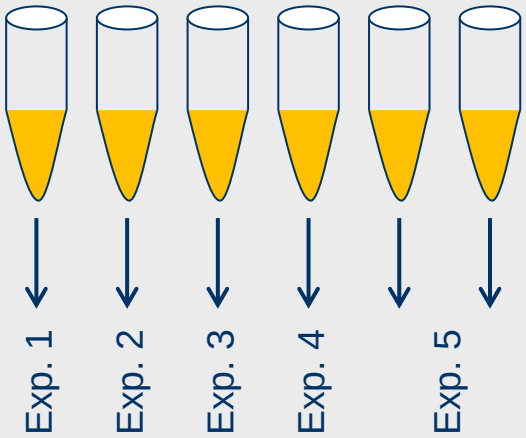
To be stored at -70°C

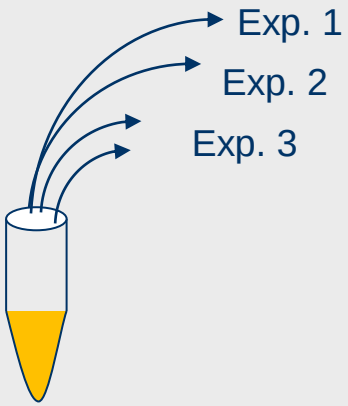
Released as reference standard. Not for human use.

06/05/2011
day/month/year Qu

Nearly all these parameters are tested during stability investigations at various storage conditions.
All together give us confidence in the stability of drug substance/product.

1. Approach: Is this practicable for each and every assay reagent too?





- Requirement of huge amounts of each reagent (single-use aliquots)
- Huge effort

- **<u>In practise</u>**
  little amounts of reagents are available only (few aliquots)
- Multiple use of a thawed aliquot (e.g. with interim storage at 4°C)
- **History of the reagent aliquot more important** for stability **than just thermodynamic stability** (e.g. traces of contamination of metal or azide can inhibit enzyme activity)
- Lot to lot differences in stability likely

Pitfalls of approach 1

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Stability of a protein is relative.

- Stability depends on concentration, buffer, temperature, vial, volume, labelling, history of handling.....
- Degradation/alteration will not affect assay performance necessarily (e.g. sandwich ELISA with excess reagents , changes in non relevant epitopes)
- Stability for use in one assay could be different from stability for another assay
- **Stability of relevant epitopes and label can be tested only by performing exactly that assay the reagents originally are intended for.**

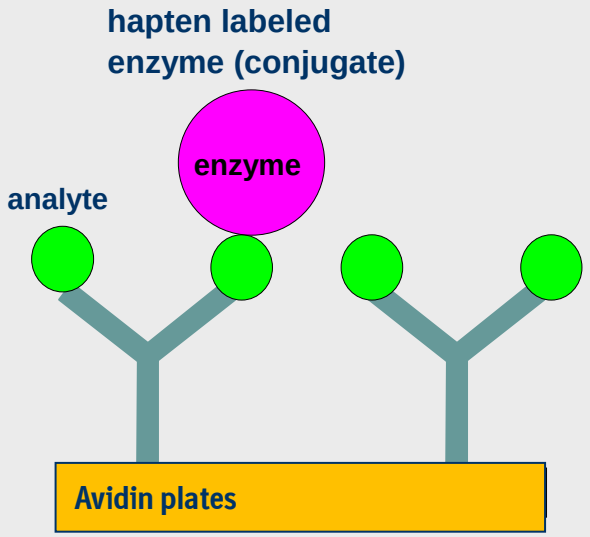
Approach 1 (comprehensive characterization of reagents) is not a feasible approach!
Go to approach 2 and 3.

2. Approach: Stability investigation by revalidation of assay (Case study 1)

- Competitive drug ELISA, developed and validated in **1993**

Assay specific reagents:

- **polyclonal rabbit anti-drug IgG** (Protein A purified and biotinylated), 30 µg/mL in PBS
- **hapten-horseradish peroxidase conjugate**, 2.5 mg/mL in PBS + 1% BSA + 50% glycerine



- Project was set on hold 1995.
  Assay reagents were stored in aliquots at **-20°C** in a monitored freezer.
- **2003** drug development re-started in a different indication.
  The old reagents were thawed and the assay revalidated.

2. Approach:
Revalidation of assay, Results (Case study 1)

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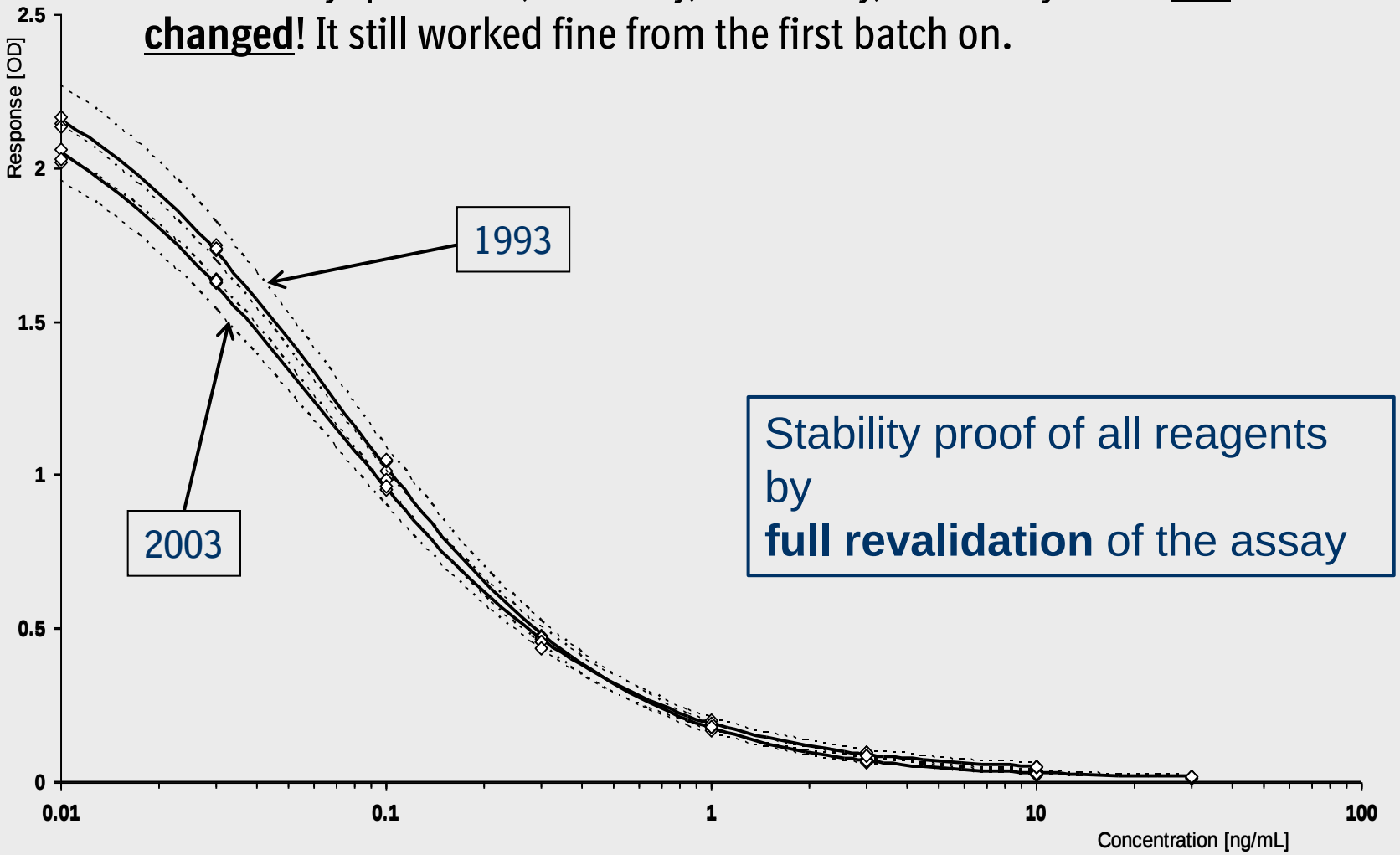
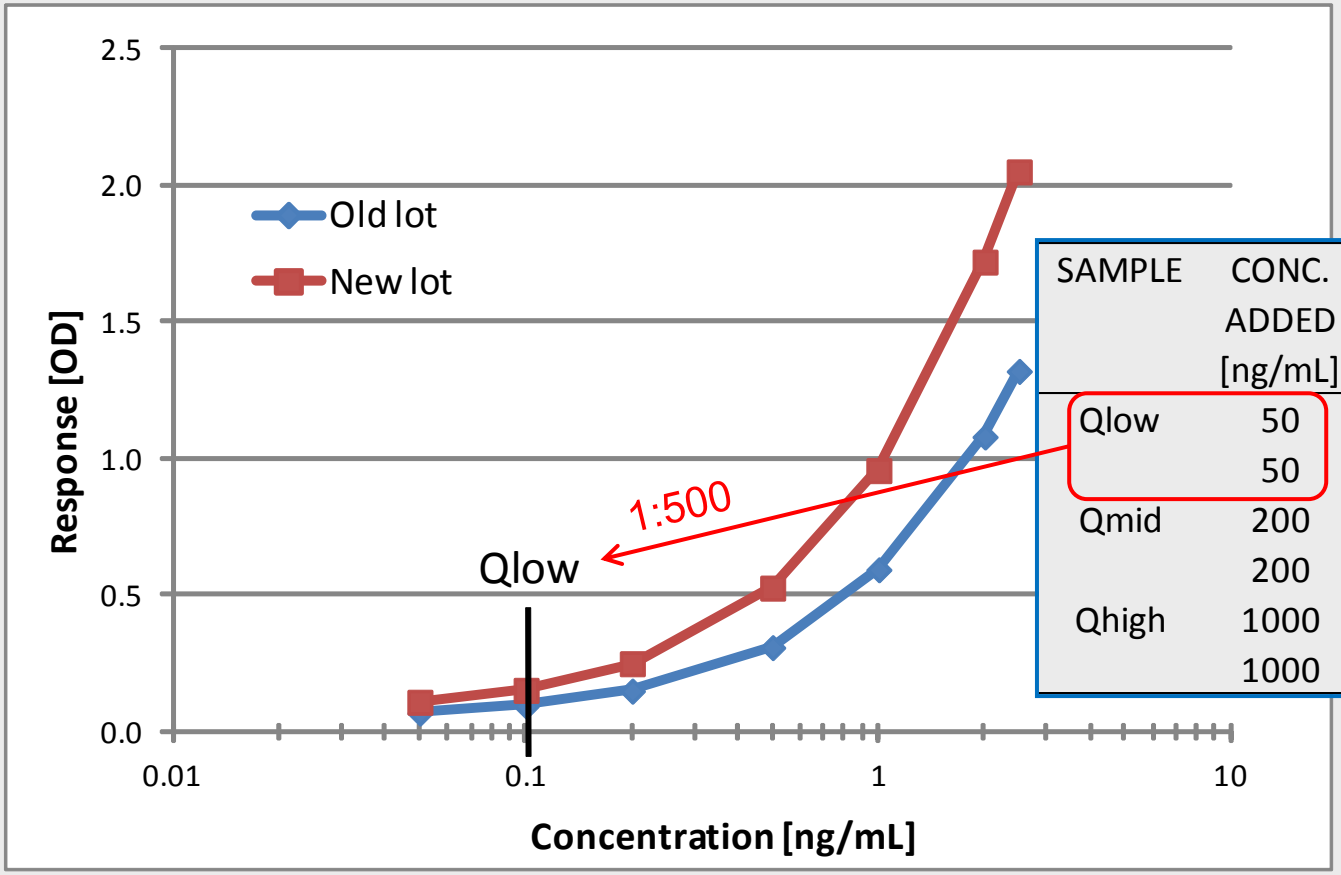
After **ten years long term storage of reagents at -20°C** the performance of the assay (precision, accuracy, sensitivity, selectivity) have **not changed**! It still worked fine from the first batch on.



Stability proof of all reagents by **full revalidation** of the assay

3. Approach: Stability investigation by batch acceptance criteria only (Case study 2)



SAMPLE	CONC. ADDED [ng/mL]	RESPONSE [OD]	CONC. MEASURED [ng/mL]	DEV. (%)
Qlow	50	0.089	43.6	-12.8%
	50	0.082	36.8	***-26.4%***
Qmid	200	0.271	211	5.5%
	200	0.270	210	5.0%
Qhigh	1000	1.180	1100	10.0%
	1000	1.150	1070	7.0%

Instability of reagent determined by batch acceptance criteria.

-> no risk to report false results

But: old reagent lot was still within the expiry date of the manufacturer!

EBF-IGM member survey „Stability of assay reagents“ 2010

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Q: Do you perform separate stability investigations, e.g. for antibodies, conjugates or do you just state: If the assay fulfils its in-study acceptance criteria all used reagents should still be OK?

- 11/12 companies do not perform additional stability investigations but rely on assay performance and in-study acceptance criteria
- 1/12 perform stock solution stability test as part of validation for reagents
- For commercial reagents: stability data/expiry dates from supplier used

EBF

Comparison of approaches

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	Pros	Cons
1. Approach **Repeated comprehensive characterization**	• Allows to identify the source of instability • scientific approach	• Huge effort • For single use aliquots only, does not cover aliquot history • Most data not relevant for just doing the assay
2. Approach **Method revalidation**	• Focus on the intended use of reagents • all assay parameter will be checked	• Several revalidations necessary (incl. specificity) • Stability proof retrospectively!
3. Approach **Batch acceptance only**	• Simple, no additional effort	• Do the batch acceptance criteria cover all aspects of reagent instability (e.g. altered selectivity/specificity)?

Case study 3: Limitations of approach 3 (batch acceptance only)

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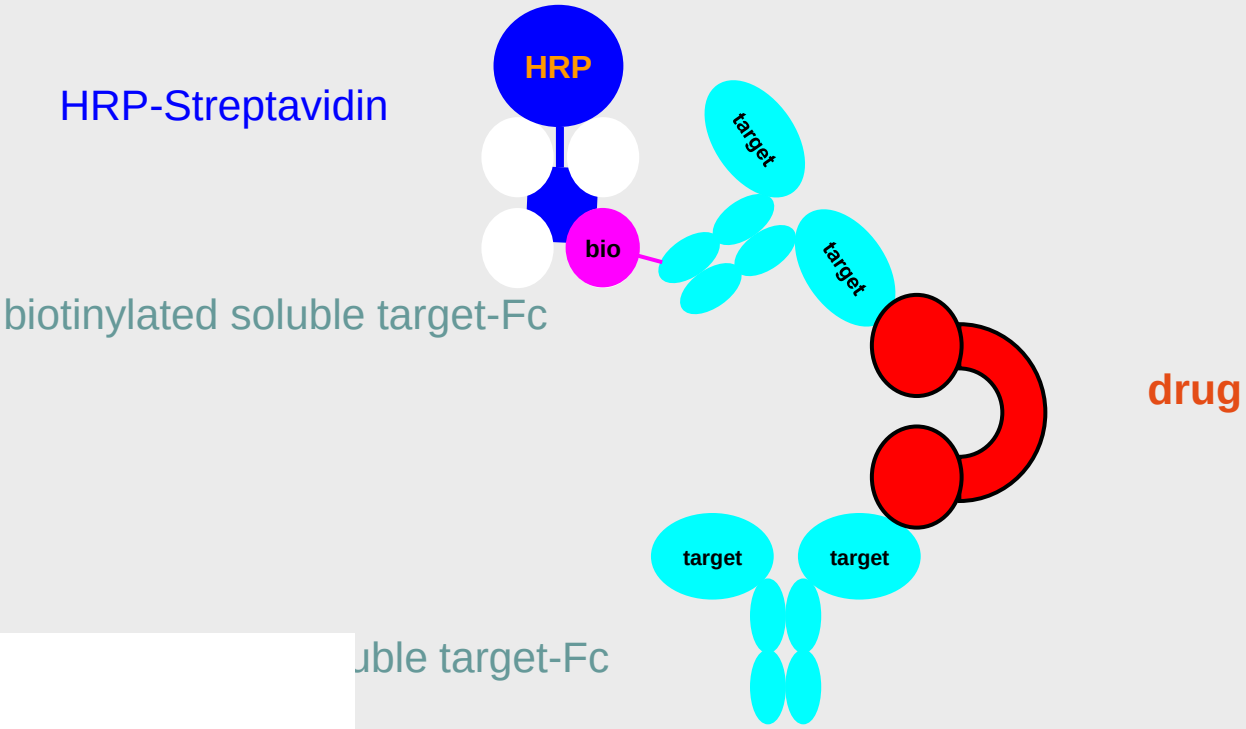
Heterogenous multi-step bridging ELISA with a high concentration hook-effect due to unstable detection reagent

Matthias Hofmann, PhD, Senior Investigator I

Novartis Biologics, Biologics Safety & Disposition, PK-PD Bioanalytics



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Calibration curve & QCs

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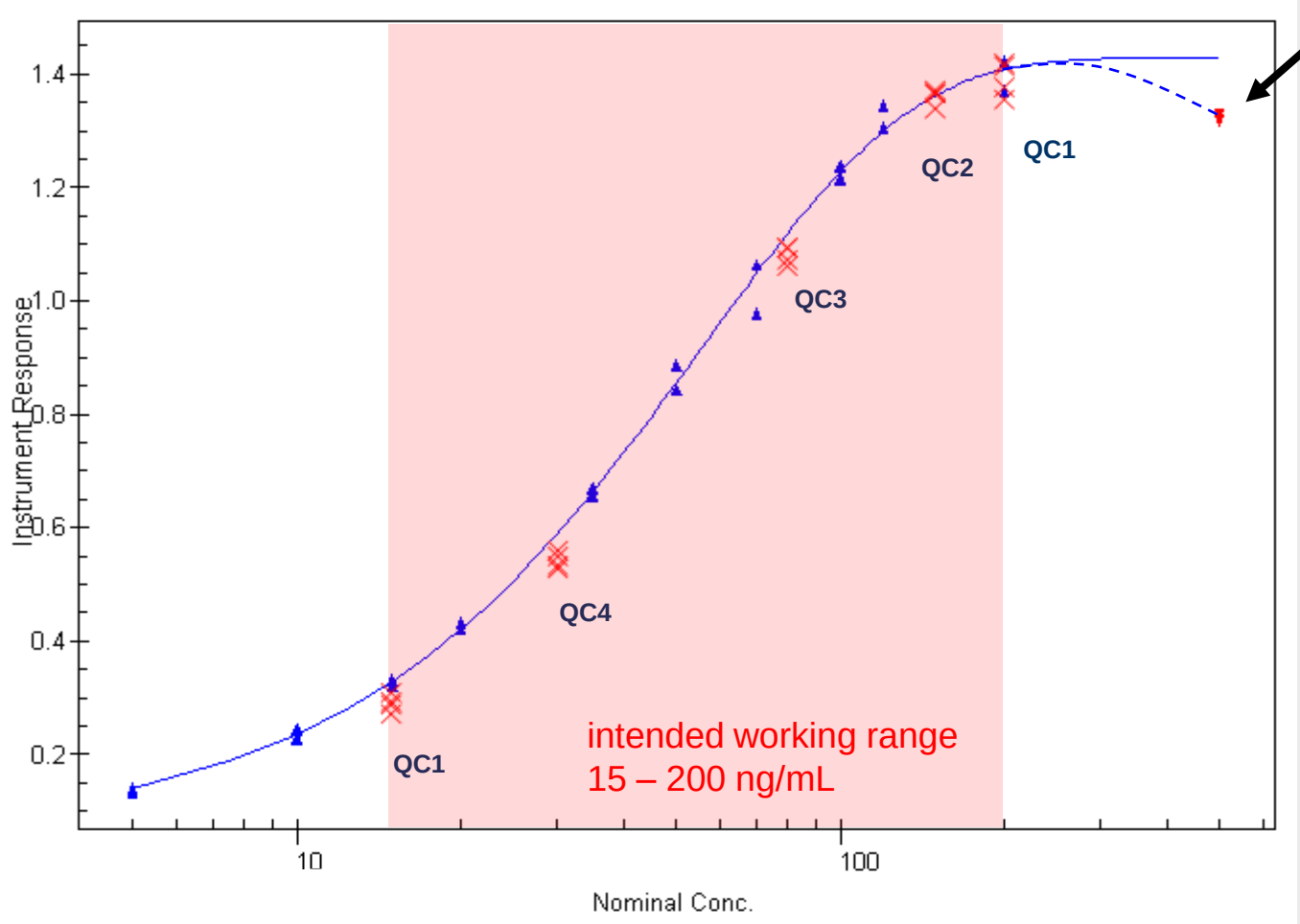
hook effect at highest calibration standard



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Hook effect related to age of detection reagent

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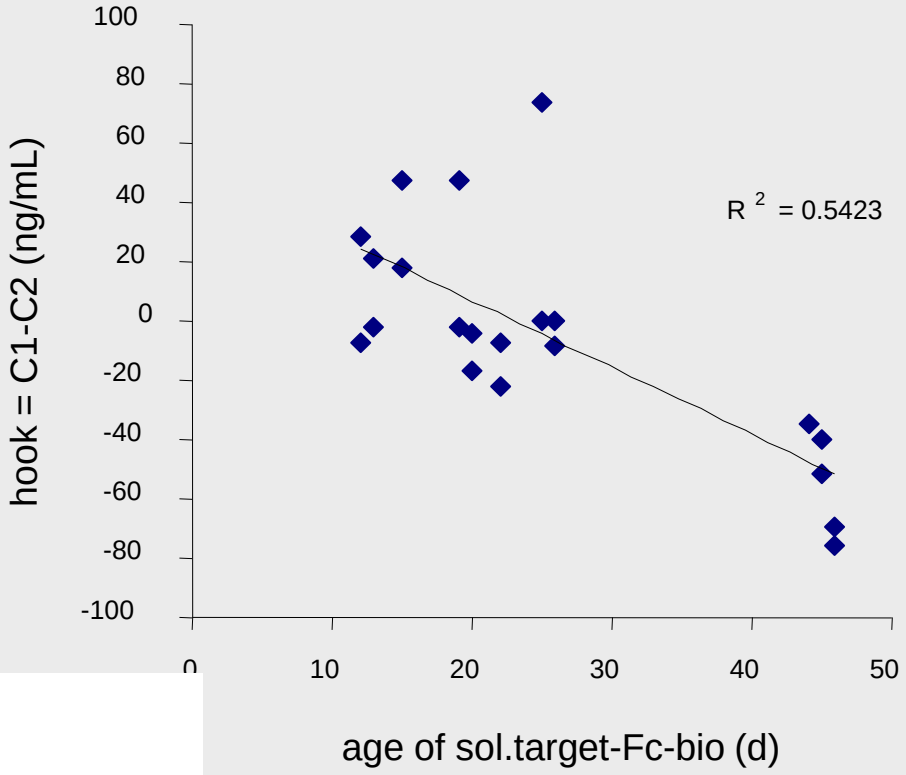
Detection reagent: biotinylated soluble target-Fc

Hook as difference of measured concentration at nominal 500 ng/mL (C1) and 200 (C2) ng/mL



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Conclusion case study 3

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- Risk to measure false too low concentrations!
- Not covered by in-study batch acceptance criteria!
- Detection reagent can only be used up to 1 month
- Assay performance needs to be closely monitored, e.g. anchor calibration point to detect Hook effect

And in general? Points for discussion

- Can we still trust in our in-study batch acceptance criteria (approach 1)?
- Routine use of anchor standards or QCs?
- Better switch to approach 2?
  (Full revalidation of assays on a routine basis, e.g. once per year, or after 3, 6, 12, 24 month)

- Does anyone have a case study in which the assay selectivity changes due to alteration of antibodies but in-study acceptance criteria and calibration curve remain unaffected?

Summary

- Experience: Many reagents (specially antibodies) can be stored for years without significant loss of activity or change in reactivity (case study 1)
- Handling (history) of reagents often more important than storage stability (case study 2)
- **Separate comprehensive stability investigations (approach 1)** of each reagent is **not feasible.**
- The stability of the reagents can only be tested by performing exact that assay they are intended for (assay specific reagent).
  - **Approach 2: Revalidation of method** from time to time
  - **Approach 3: Trust in the in-study batch acceptance criteria as a proof for reagent stability (still the most favored and practicable approach)**
- There could be cases of instability that are not coverd by the in-study batch acceptance criteria (case study 3). **These have to be discussed!**

Acknowledgement

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