Stability of anti-drug antibodies in human samples

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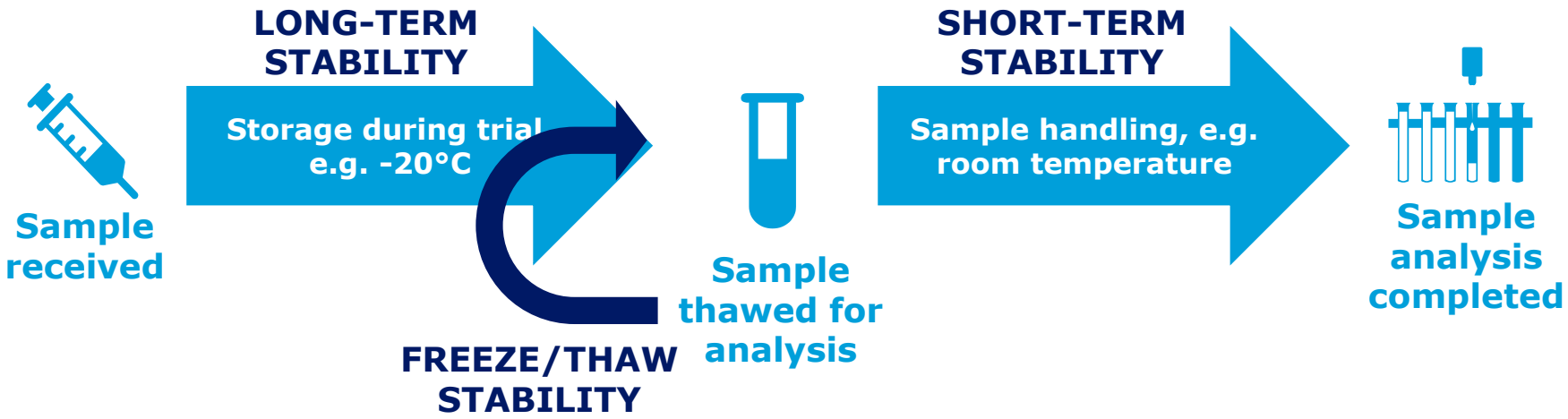
Agenda

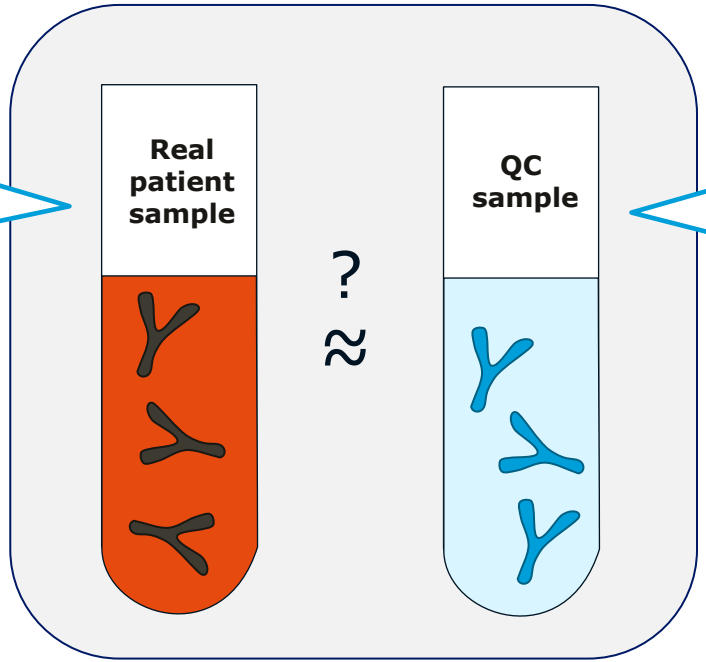
1. Assessment of stability according to **guidelines/white papers**
2. Assessment of stability in **literature/CROs**
3. Assessment of stability at **Novo Nordisk**
4. Question from regulators regarding stability testing
5. Stability assessment on Novo Nordisk going forward

Defining stability



Testing stability of ADA or control?

True ADA analyte
- Polyclonal
- Human origin



Surrogate for the ADA analyte
- Polyclonal or monoclonal
- Animal origin

ADA: Anti-drug antibody

Stability assessment from guidelines

- FDA Draft Immunogenicity Guidance, 2016
  - *No specific recommendations on antibody stability testing*
  - Sponsor **avoid freeze-thaw** cycles
  - May be useful to evaluate **long-term stability** of positive control antibodies
  - For more information on stability studies, see the guidance for industry Bioanalytical Method Validation

- FDA Bioanalytical Method Validation Guidance, 2018
  - *No recommendations on antibody stability testing*
  - Stability of the **analyte** is evaluated using low and high level QC samples
  - Stability should cover **short-term stability** at room temperature or sample processing temperature and **freeze-thaw stability**
  - **Long-term freezer stability** should be studied at each temperature at which study samples will be stored

Stability assessment from guidelines

- EMA Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins, 2017
  - *No recommendations on antibody stability testing*

- USP 1106 Immunogenicity Assays
  - For samples stored at or below **−20°C**, the stability of ADA are **universally accepted**, so this sample storage condition may not require validation
  - It is generally accepted that an ADA sample in serum or plasma will be stable after **three freeze–thaw cycles**

Stability assessment from white papers

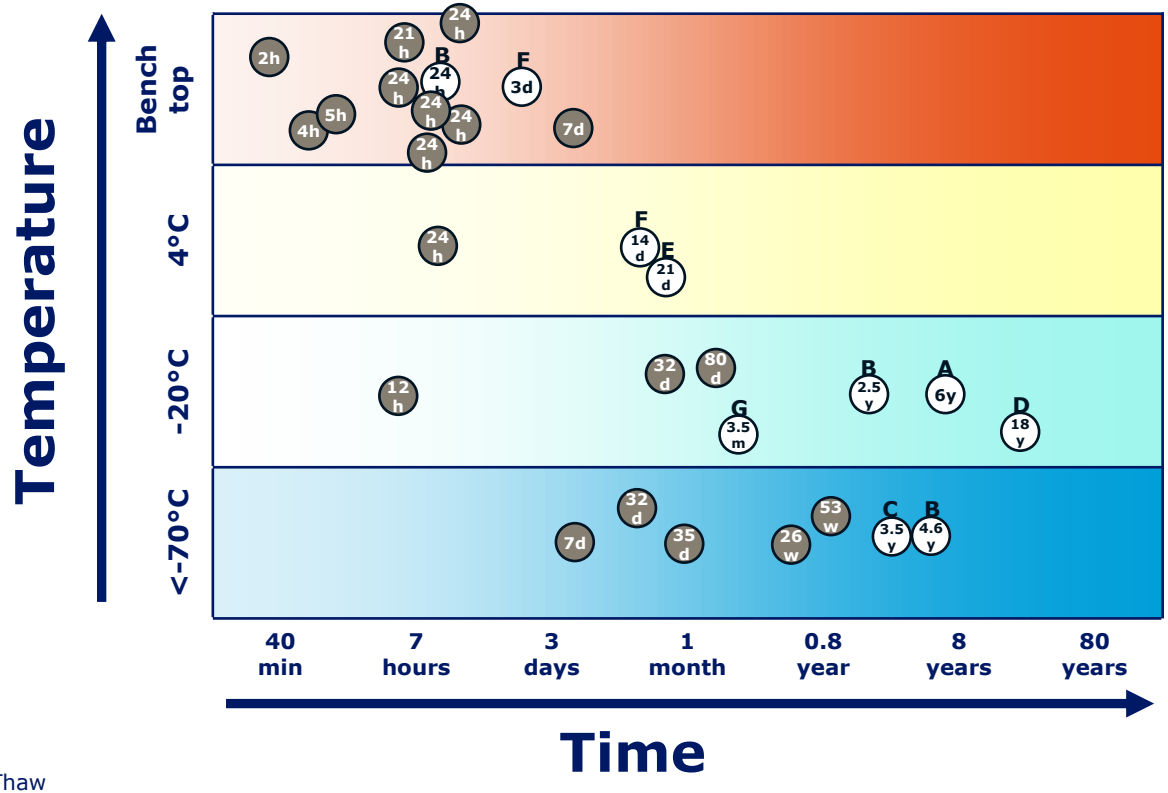
- Shankar, 2008
  - ADA stability is the same whether it is specific to drug X or drug Y
  - → ADA stability can be approximated by the stability of serum or plasma Ig specific to **any** antigen

- Bioanalysis 2018[1]: Focus workshop, European Bioanalysis Forum
  - Since it is well known and documented that antibodies are stable in serum and plasma matrices, the recommendation was to **use trending analysis of positive controls** as a viable alternative to formal stability assessments.

- Conclusion from guidelines/white papers regarding antibody stability
  - **No formal/aligned recommendation exists**

[1]Joanne Goodman et al. **Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles**. Bioanalysis. 2018 Jan; 10(4):197–204

Mapping of accepted stability studies



Stability papers (Patient samples)

A. Solberg, 2010 (**4 F/T**)
B. Hendriks, 2014 (**8 F/T**)
C. Michaut , 2014 (**12 F/T**)
D. Castejon, 2014
E. Demir, 2014 (**5 F/T**)
F. Bartholdy, 2015
G. Castejon, 2017 (**174 F/T**)

CRO studies (**3-8 F/T**) (QC samples)

F/T: Freeze/Thaw

EBF Survey: inclusion of stability studies[1]

In total, 27 companies responded (anti-drug antibodies and anti-vaccine antibodies)

- All responders use spiked QC samples for stability testing

Response	Short-term	Long-term	Freeze/thaw
Yes	70%	59%	95%
No	30%	41%	5%

- No long-term instability observed (>55 different biological drugs included)
  - → Long-term stability not necessary

- No short-term instability described
  - Bench-top storage of the samples at 4°C or ambient, as well as freezing and thawing them, represent more stressful conditions for the samples
  - → May be relevant to include short-term stability (freeze/thaw) testing

[1]Pihl L, Hendriks J, Loebbert R, Ryding J, Nemansky M, Vermet L, Companjen A. **EBF recommendation for stability testing of anti-drug antibodies; lessons learned from anti-vaccine antibody stability studies**. Bioanalysis. 2014 May;6(10):1409-13.

Previous Novo Nordisk strategy on stability testing

- No project specific stability testing done

- Referring to literature and internal stability studies
  - No specific stability section in the validation report
  - Long-term, short-term and freeze/thaw stability not clearly described

  → difficult for a reader to see if stability had been assessed at all

- No trending of QCs

Responses from regulators regarding stability (case 1)

Question (modified)

- You did not provide information to support the **stability of your samples**
- Ensure the **freeze/thaw** stability of QC antibodies is adequate to meet testing needs

Novo Nordisk answer (modified)

- The QC trending demonstrates a consistent readout
- Stability of antibodies has been shown for at least 8 times freeze/thaw cycles and at least 5 years at -20°C
- It is generally recognized that the stability of the antibodies is independent of the specificity of the antibody
- Two additional studies analysing stability of antibodies against other protein therapeutics supports the published literature on this topic (*internal studies*)

Responses from regulators regarding stability (case 2)

Question (modified)

- You did not provide data demonstrating the stability of the positive control antibodies
- In order to demonstrate that the antibodies remain stable under normal testing conditions assess the performance of the antibodies under **long-term storage, freeze-thaw, and benchtop conditions**

Novo Nordisk answer (modified)

- In seven trials, the positive control antibodies were shown to be stable during long-term storage for at least up to 18 months at -20°C
- The validation data shows that the antibodies are stable for at least 3 freeze-thaw cycles
- The validation results demonstrate stability at benchtop conditions for up to 24 hours

What do the regulators want?

- Do the regulators request project specific stability testing?

- Do the authority just request sufficient scientific explanation?
  - Justifying why stability testing is not done
  - Describing how stability can be evaluated from the QC trending
  - Referring to all the data described in the literature

Novo Nordisk internal stability studies

Long-term and freeze/thaw stability

76 patient samples were:

- Stored at -20°C
- Thawed and frozen 4 times over 6 years
- Analysed 4 times during this period

Short-term stability (to resemble sample handling)

12 patient samples were:

- Stored at room temperature for 24, 48 and 72 hours
- Stored at 4°C for 7 and 14 days
- Snap frozen at dry ice prior to freezing at -20°C

Results from long-term and freeze/thaw stability study

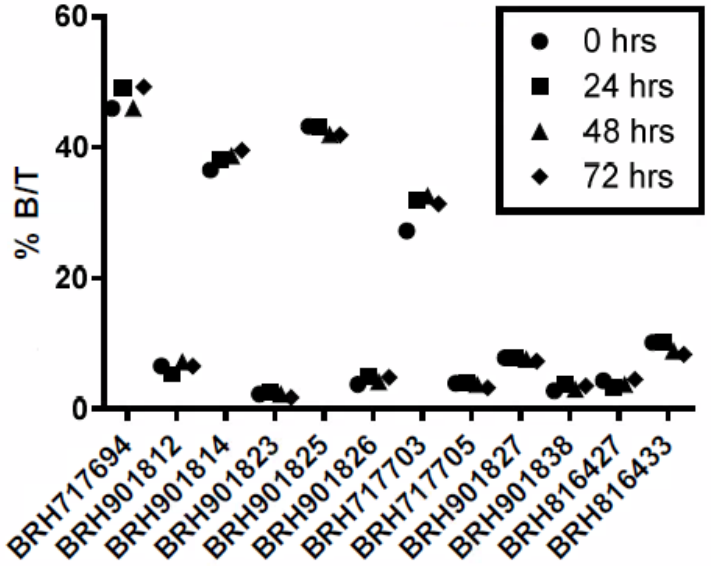
All samples freeze/thawed (up to 4 times)	Mean difference between first and last analysis (%B/T)[1]
Samples stored for 2½-4 years (N=16)	1.1
Samples stored for 4-5 years (N=39)	-3.0
Samples stored for ≥ 6 years (N=21)	-0.1
All samples	-1.3

[1]: Mean of first sample analysis, %B/T = 27 [ranging from 0.1 to 65.5]

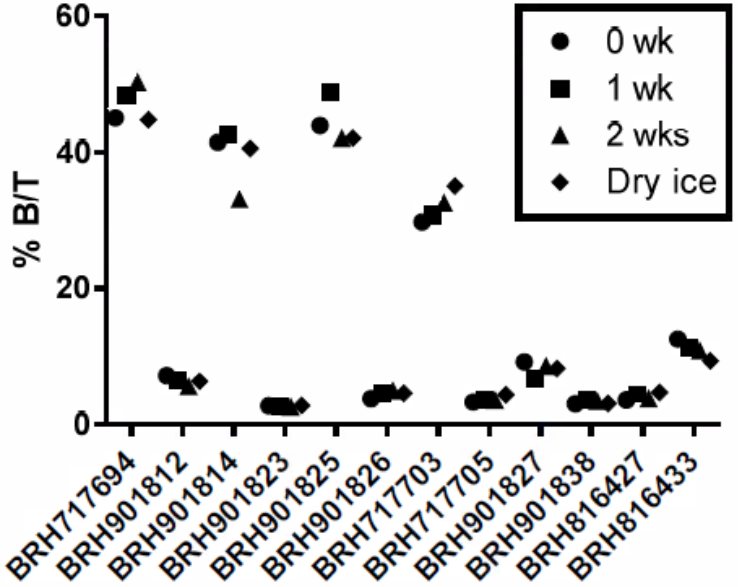
Immunogenicity Assessment

Results from short-term stability study

Room temperature (22°C)



4°C or dry ice



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Conclusion on internal stability studies

- Human serum antibodies are stable for
  - At least 4 freeze/thaw cycles
  - At least 6 years at -20°C
  - At least 2 weeks at 4°C
  - At least 72 hours at room temperature
  - Freezing on dry ice for 4 hours

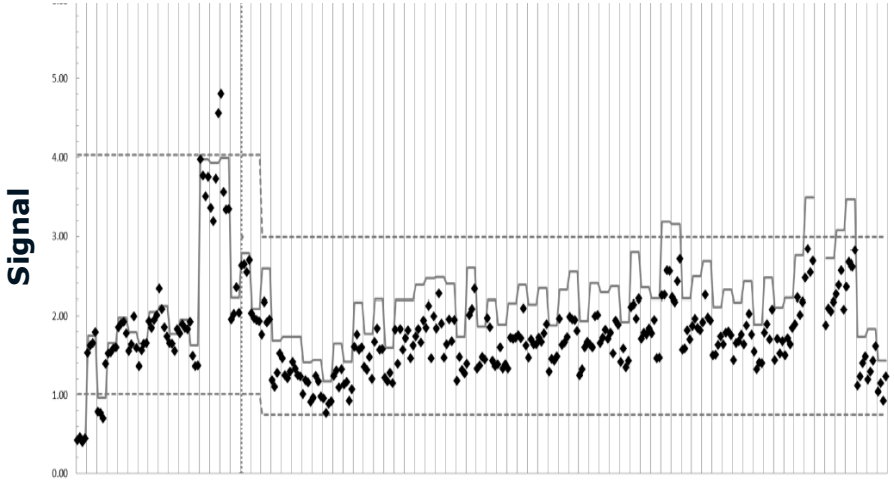
Novo Nordisk: Validation stability section

- Antibodies are generally considered to be very stable when stored at or below **-20°C** (1, 2)
- Long-term stability of antibodies has been demonstrated for a minimum of **6 years** (2) and upon up to **4 repeated freeze/thaw cycles** (2, 3)
- Furthermore, short-term stability of antibodies has been demonstrated for minimum **72 hours** at room temperature (**22°C**), for minimum **2 weeks** at **4°C** and after freezing on **dry ice** for minimum **4 hours prior to storage at -20°C** (4)
- It is assumed that the stability of ADA is independent of specificity (5) and therefore the stability of NNCxxxx-xxxx specific antibodies will not be tested in this validation
- As part of assay life-cycle management and as a viable alternative to formal stability assessments, collecting and trending data on QC samples will be performed (6).

1. United States Pharmacopea. Immunogenicity Assays — Design and Validation of Immunoassays to detect Anti-drug antibodies, 1106: 1382-1397.
2. Solberg H. 2010. Long Time Stability of Human Antibodies in Serum Stored at minus 20°C. Internal Novo Nordisk report, study no.: 207160, novoDOCS ID: 539767.
3. Hendriks J et al. 2014. Stability studies of binding and functional anti-vaccine antibodies. Bioanalysis, 6(10): 1385-1393.
4. Bartholdy C. 2015. Stability evaluation of human antibodies in serum. Internal Novo Nordisk report, study no.: 215020, novoDOCS ID: 002416733.
5. Shankar G et al. 2008. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. Journal of Pharmaceutical and Biomedical Analysis, 48, 1267–1281.
6. Goodman J et al. 2018. Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles. Bioanalysis. 2018 Feb;10(4):197-204. doi: 10.4155/bio-2017-4971. Epub 2018 Jan 18.

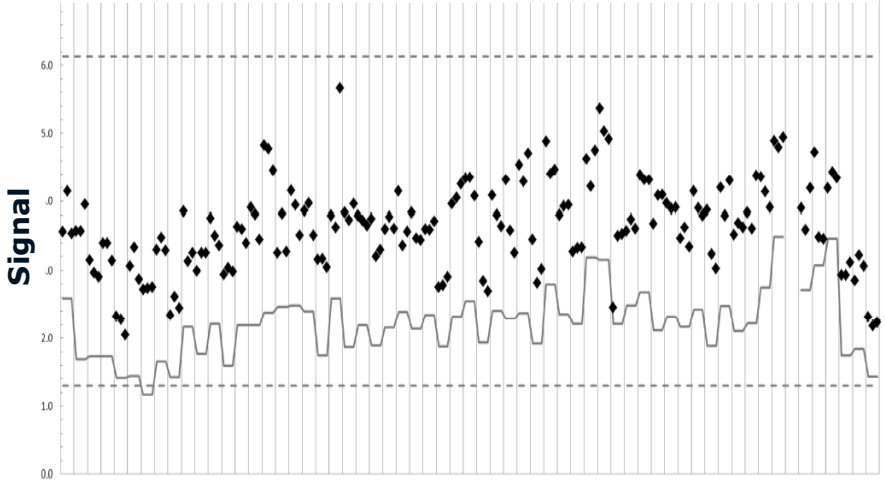
Trending of QCs

QC negative



Individual set-ups over time

QC positive



Individual set-ups over time

Suggested approach for stability assessment

- **No project specific stability studies will be performed**
  - Literature, industry and internal data support freeze/thaw, short- and long-term stability of antibodies
  - Trending of QC samples ensures stability of the control antibodies

- **Have a clear description in the validation report that justifies why stability experiments are not necessary**

→ Risk that regulators may request additional stability data

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