SESSION VI

Quality Control & Stability Testing for Investigative Medicinal Product (IMP) and Registered Drugs

Commonwealth Scientific Research organisation (CSIRO)

Australian national science agency

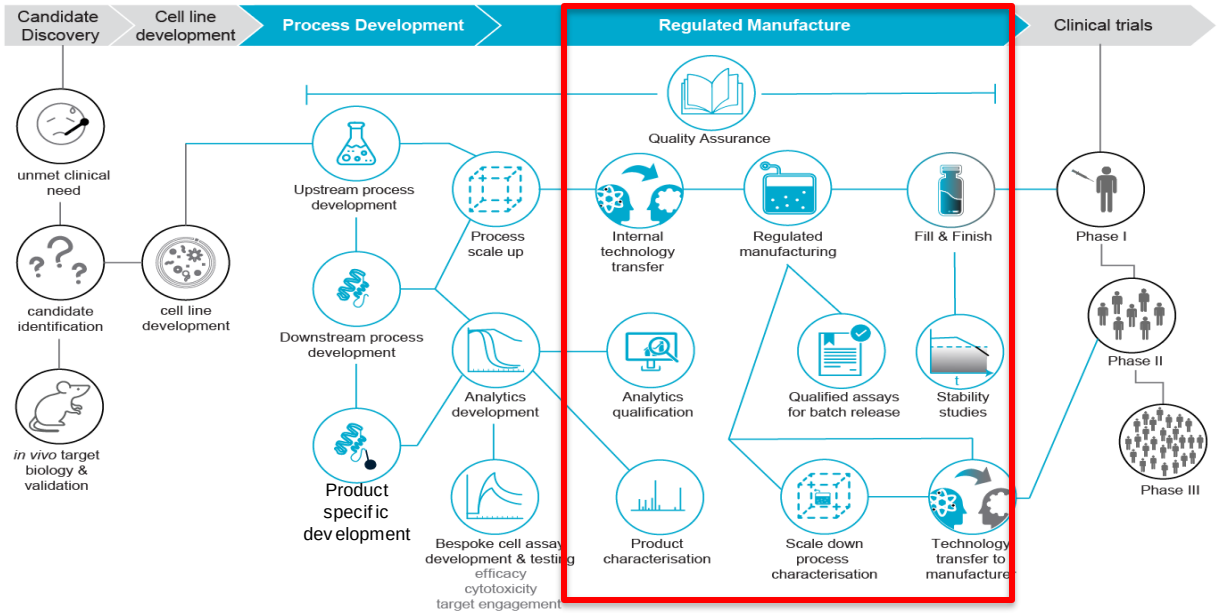
- 5,500 dedicated people
- 51 sites across the world including Australia, Singapore, Vietnam, US and Chile
- $4.5B+ a year value delivered to the Australian economy through our science and technology
- One of the world's largest multidisciplinary science and technology organisations

Key areas of research

- Animals and plants
- Astronomy and space
- Climate
- Environment
- Farming and food production
- Health
- Information technology
- Mining and **manufacturing**
- Renewables and energy

CSIRO's mission is to create benefit for Australia through science and innovation, and supportting Australian industry.

CSIRO Biomedical Manufacturing



To bridge the gap between benchtop lab research and commercial scale by providing local manufacturing infrastructure (especially in the biologic sector) to the local industry and the chance to compete globally.

National Vaccine and Therapeutic Laboratory (NVTL)

Application Areas:

Mammalian cell-based vaccine and drug candidates manufactured in large quantities for Phase I clinical trials.

Upstream Production:

- Cell seeding, inoculation to harvest
- Scale up from benchtop (< 10 L)
- 50 L & 200 L scale working volumes

Downstream Production:

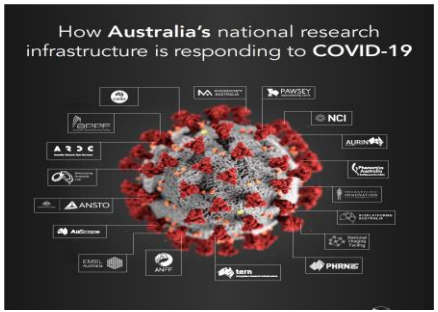
- 10 L to 200 L protein purification
- Clarification & purification to Drug Substance (DS)
- Formulation to Drug Substance (DP)

QC Analytics:

- Assays designed for product release specifications

Fill & Finish:

- Dispensing into various vial sizes and presentation
- 100% visual inspection to USP <1790> standards
- Packing and labelling



Case Study: Phase 1 Drug Product through Analytical Testing

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Regulated Biomanufacturing | CSIRO

2023 PDA Asia Pacific Regulatory Conference

Outline

- Introduction
- Case study – Sequence Variants
- Case study – HCPs
- Summary

Primum non nocere

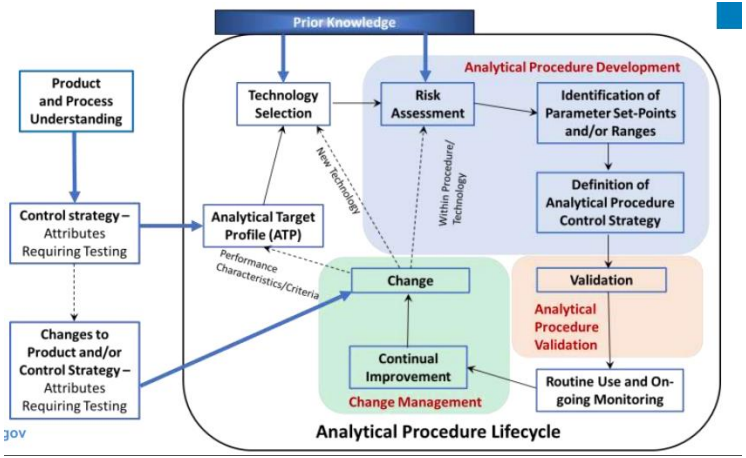
- We all came to science through different drivers.
- However, as those tasked with shepherding novel therapies to age old diseases, we must hold one thing above all else:

First, do no harm

Or as I see it, safety first always!

Introduction

- The identification, development and conduct of analytical testing of Investigation Medicinal Products (IMPs) is guided by multiple regulatory documents including but not limited to ICH Q6B, ICH Q2(R2), ICH Q14.
- These documents outline/suggest the key characteristics that need to be assessed and monitored during the product and clinical development of products.
- Additionally, prior knowledge from the field and from other comparator molecules help set boundaries and expectations.



Ref: ICH Q14

Introduction

- Assays can be broken down into the characteristic they are informing.
  - General i.e. colour, clarity, pH
  - Strength i.e. API concentration
  - Identity i.e. sequence order of amino acids or key biophysical characteristic (PM, charge, etc.)
  - Purity i.e. presence of in-process or product related impurities
  - Potency
  - Safety i.e. bioburden, sterility or endotoxin
- During product development and pre-clinical studies, we are selecting the assays to conduct to generate the data.
- This early data on the biologic will lead to the specifications set for Ph 1.
- As we usually have limited samples, it is important to maximise the information we draw from these sources.
- In this presentation, I will focus on the importance of an Identity test and the power it can bring to Purity.

Identity

- "*The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory.*" (FDA, 21CFR610.14)
- The development and qualification of these assays are critical to ensuring we have a fingerprint of the reference samples to compare to subsequent batches for Ph 1 clinical trials.
- For biologics, these can include the peptide sequence, their charge profile or their glycosylation pattern (N/O-linked).
- For biologics, the sequence or order of amino acids is unique and critical to function.

Peptide Mapping

- The gold standard for probing the sequence identity of biologics is peptide mapping.
- This process involves:
  - The selective cleavage of the proteins into predictable peptides.
  - Separation by reverse phase chromatography.
  - Detection either by a UV detector alone or coupled to a mass spectrometer.
- As the biophysical properties of peptides differ considerably due to their amino acid components, no two biologics will share the same fingerprint (unless they have the exact same sequence).

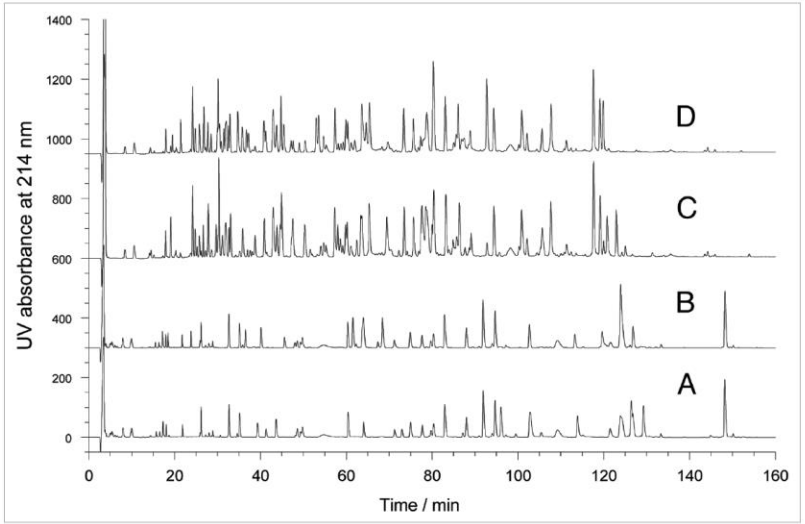
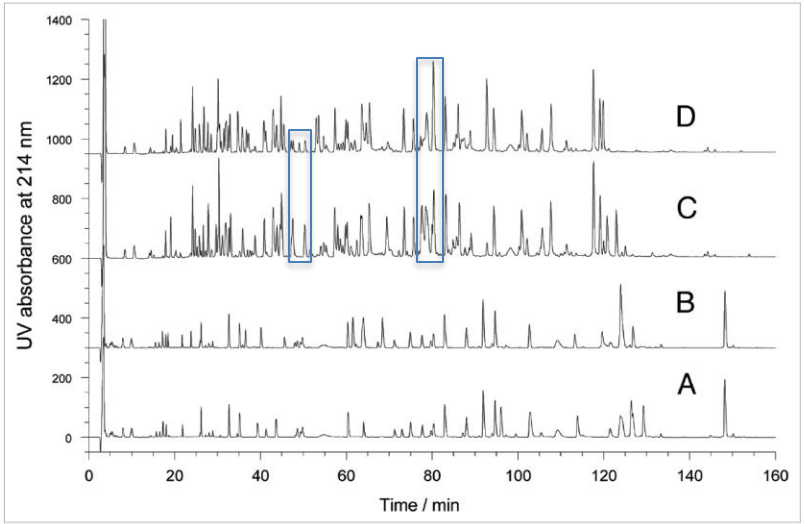


Figure 1. Full view of the HPLC-UV peptide maps of rhumAb HER2 and rhumAb A. (A) Tryptic map of rhumAb HER2; (B) Tryptic map of rhumAb A; (C) Chymotryptic map of rhumAb HER2; and (D) Chymotryptic map of rhumAb A.

Ref: Yang Y.et al. (2010) mAbs 2(3):285-298

Peptide Mapping

- As the biophysical properties of peptides differ considerably due to their amino acid components, no two biologics will share the same fingerprint (unless they have the exact same sequence).
- Differences observed between two different batches of the same antibody could indicate the presence of modifications.
- These differences provide critical information on the robustness of the process, and potentially on the stability of the sample.
- Careful interrogation of the data can also provide indicators to the presence of impurities in the samples.



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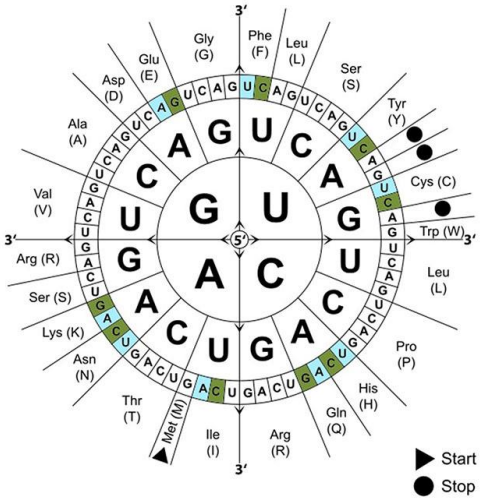
- Case study – Sequence Variants
- Case study – HCPs
- Summary

Case study – Sequence Variants

- The central “dogma” of molecular biology states that DNA is transcribed to RNA and then subsequently translated to protein.
- The triplet codon code is well understood, and rare codons are known.
- Therefore, constructs are designed to avoid use of rare codons.
- The assumption is then that the protein sequence generated should match the DNA sequence inserted.

A



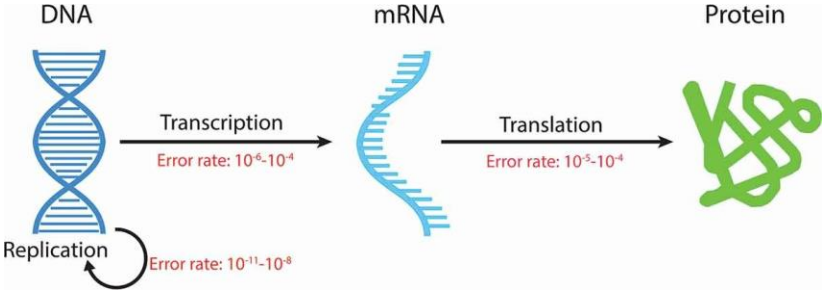
B

Amino acid	Codon	P. patens	A. thaliana	S. cerevisiae	S. pombe	H. Sapiens
Cys	UGC	+	+	-	+	+
	UGU	-	-	+	-	-
Glu	GAA	-	-	+	-	-
	GAG	+	+	-	+	+
Phe	UUC	+	+	+	+	+
	UUU	-	-	-	-	-
His	CAC	+	+	+	+	+
	CAU	-	-	-	-	-
Ile	AUA	-	-	/	-	-
	AUC	+	+	/	-	+
	AUU					
Lys	AAA	-	-	-	-	-
	AAG	+	+	+	+	+
Asn	AAC	+	+	+	+	+
	AAU	-	-	-	-	-
Gln	CAA	-	-	-	+	-
	CAG	+	+	+	-	+
Tyr	UAC	+	+	+	+	+
	UAU	-	-	-	-	-

Ref: https://en.wikipedia.org/wiki/Codon_usage_bias

Case study – Sequence Variants

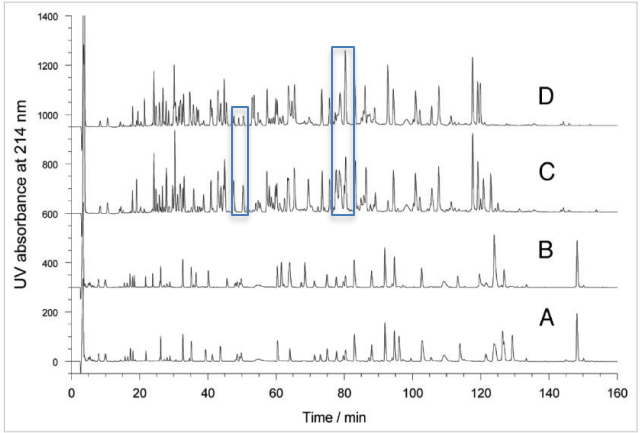
- What is often lost is the error rate of each step.
- These errors lead to what is now referred to as sequence variants.
- Early reports of sequence variants can be found starting in the early 2010s.
- These were initially observed as low-level species during monoclonal characterization.



Ref: Zhang A et al. (2020) mAbs 12(1):1791399

Case study – Sequence Variants

- Careful interrogation (presented on Slide 9) of the data by Yang and colleagues identifies an additional peak in one of their clones.
- The new peak was not present in three other samples of the same antibody and was observed to be eluting earlier than a known peak.
- Early eluting peaks could represent a post translational modification like oxidation.
- However, UV alone is not sufficient to identify this peak.
- For that the authors turned to mass spectrometry.



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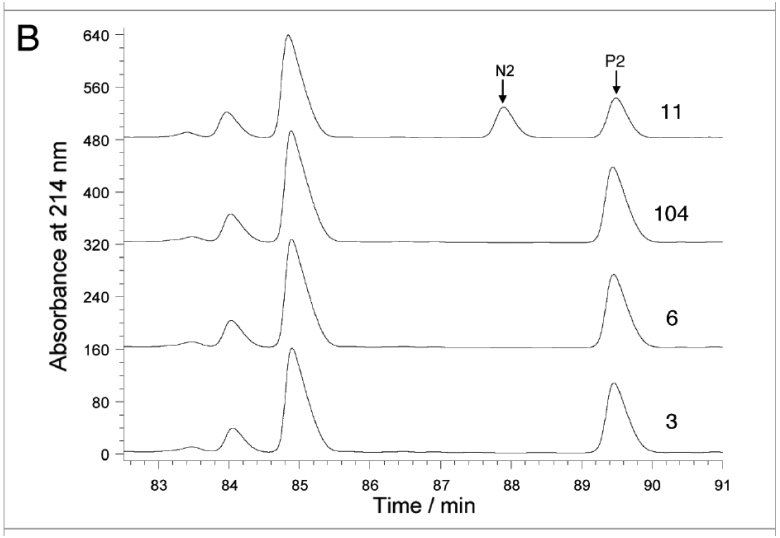

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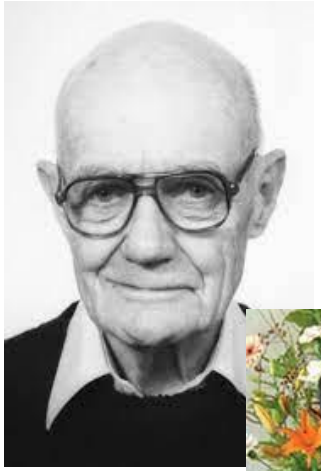
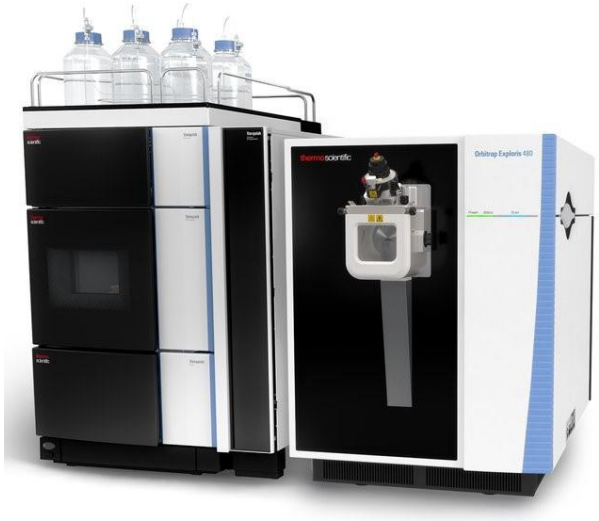
Introduction – Mass spectrometry

- At its simplest, mass spectrometry (MS) is the measurement of mass/charge (*m/z*), as opposed to spectroscopy which is measurement of light.
- Analysis requires the transfer of material from solid/solution phase into the gas phase.
- Modern biological mass spectrometry draws on work by the 2002 Nobel laureates Fenn and Tanaka - with key contributions from Karas and Hillenkamp.
  - These scientists were the first to describe soft ionization which enabled the analysis of biological compounds.
  - Prior to the work by these scientists the ionization of compounds were too harsh to maintain the integrity of proteins, lipids, carbohydrates and nucleic acids.



Introduction – Mass spectrometry

- Coupled to a LC unit, such as that shown on the right, mass spectrometers can measure the mass of all the peptides within any peak.
- This mass is unique to the composition of amino acids present in the peptides.
- To determine the order of amino acids, and thus the pepdites identity, any peptide can be selected and fragmented by collision into the background gas inside the mass spectrometer.
- This collision breaks apart the peptide and the sequence can be read from either termini.
- This sequence can then be compared to the API sequence or databases to identify the source of the peptide.



Sequencing

- Sequencing of the peak labelled P2 identified the sequence as expected.
- Sequencing of the peak labelled N2 identified a peptide with a P274T mutation.
- This mutation is unlikely to be caused by sample handling (i.e the experimental procedure used to generate the data).
- Thus the most likely source of the mutation is either at the during transcription/translation.

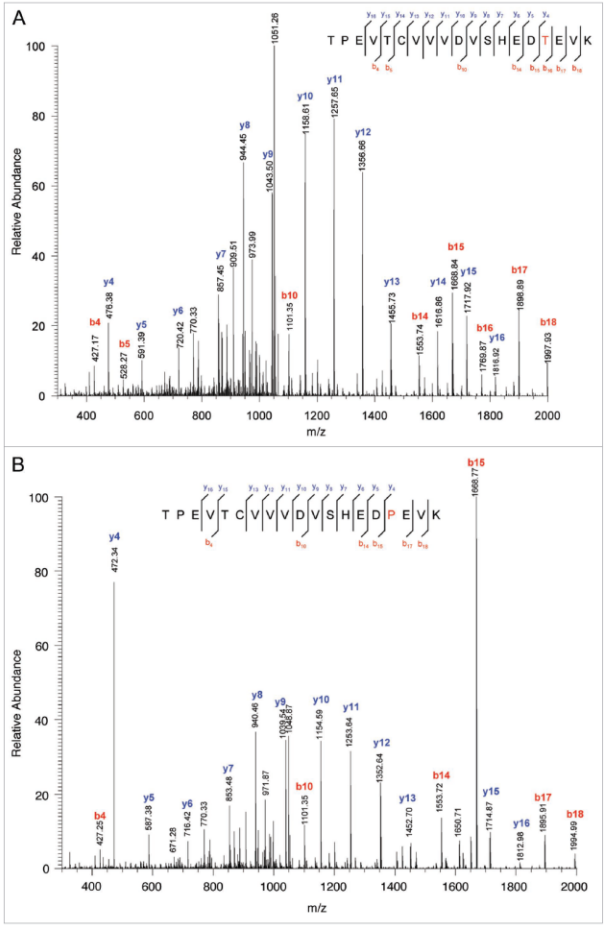


Figure 6. MS/MS spectra of the variant peptide (A) and the expected peptide (B) of rhumAb B from clone #11.

Ref: Yang Y.et al. (2010) mAbs 2(3):285-298

Sequence Variants

- One of the most common causes of sequence variants involves the misincorporation of amino acids during translation.
- One common cause is the lack of the relevant amino acid in the cell, leading to a “mischarging” of the tRNA.
- Work by Feeney and coworkers on a tyrosine sequence variants demonstrated the impact of the addition of tyrosine to the feed.
- As the levels of tyrosine decreases in culture, there is a corresponding increase in sequence variants cause by the replacement of tyrosine by other amino acids.
- The occurrence of these sequence variants reach as high as 1.2% over the course of a standard cell culture process (14 d).

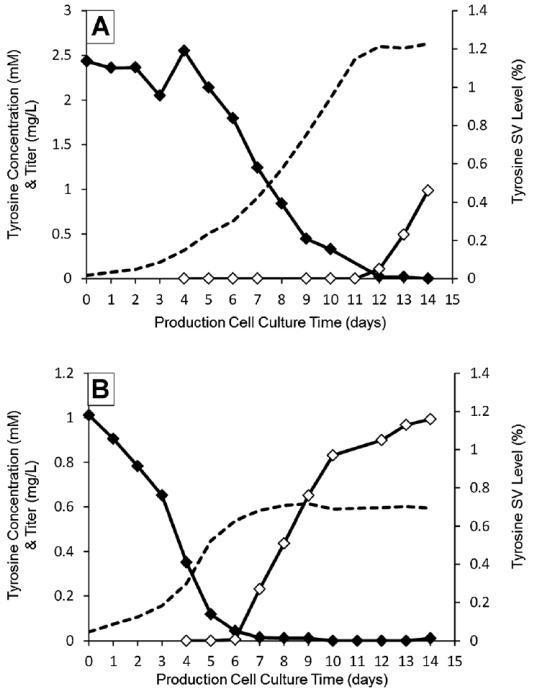


Figure 4. Relationship between tyrosine concentration and SV formation for mAb 1. Tyrosine concentration (◆), time course SV formation (◇), and titer (----). **A**: Control case (no extra tyrosine supplementation); (**B**) Early in culture tyrosine starvation case.

Ref: Feeney L.et al. (2013) Biotech and Bioeng 110(4):1087-1097

Sequence Variants

- What impact could these changes have?
- Some possible examples:
  - A mutation in CDR3 of the HC could alter the binding to its antigen.
  - A mutation in the hinge replacing a Cys, could destabilise the antibody.
  - A mutation from N->D could lead to an aglycosylated antibody, directly impacting stability of the antibody.
  - A mutation to a catalytic triad amino acid, such as S -> A would inactive the enzyme.
  - More broadly, changes in amino acid sequence could also lead to increase immunogenicity.
- Detecting and eliminating these variants before the process is locked for Ph 1 is critical to safety.
- Finally, this ensures that the specifications set for identity (i.e. Comparable to reference) is accurate and appropriate.

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Case study – HCPs

- CHO cells are a favourite cell line for the generation of many biologics.
- A key in-process analytical tool is the determination of the levels of host cell protein (HCP) in relation to the API.
- Routinely, many labs rely on commercial kits such as those provided by Cygnus or Cytiva to monitor the reduction in HCP at each unit operation.
- However, these kits are generic and may not always detect proteins specific to your MCB and process.

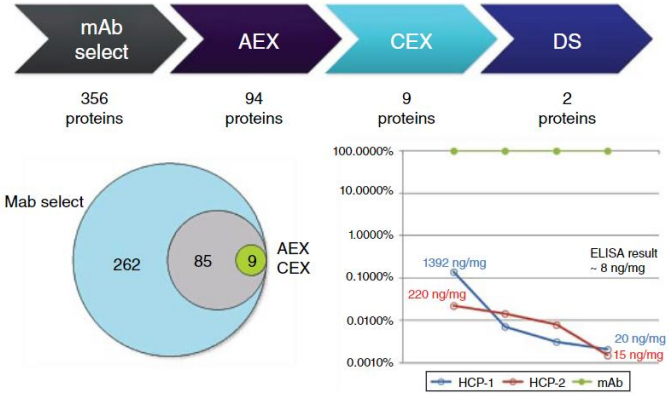


Figure 10.5 Assessment of HCP clearance during the purification of a mAb. HCPs are detected and quantitated for each process pool and the final drug substance.

Ref: Krawitz D. et al. (2017) Analytical Characterization of Biotherapeutics, 211-237

Case study – HCPs

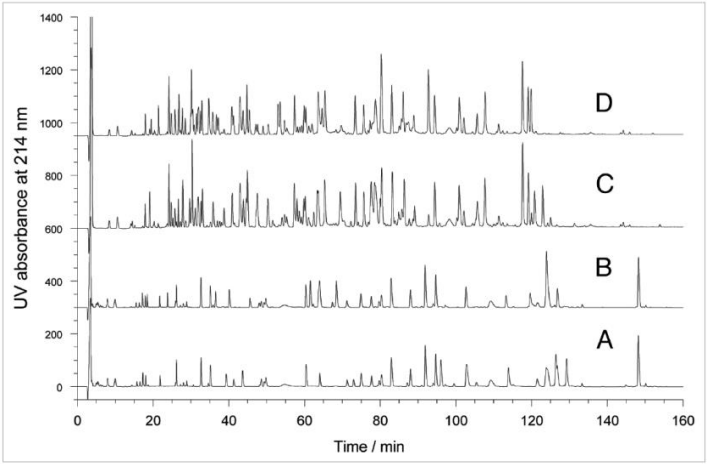
- An alternative to the ELISA is peptide mapping with an in-line MS, however the key challenge was detecting proteins 6 orders of magnitude lower in abundance than the main species.
- In a final down stream process, HCP levels can start at 1,000s ppm (part per million) and reach sub-ppm at the end.
- Thus the method has to be robust enough to measure across this range.
- This dynamic range and the adapting of the peptide map assay can play a critical role in the ability to detect HCPs in the presence of the API.
- Work by multiple authors such as Sperry and co-workers at Pfizer and Chen and coworkers at Waters identified unique methods to address these issues.

Dynamic Range

- As described previously, the sequence identity of peptides requires a unique step of events to occur.
- Regardless of the operating mode of the mass spectrometer, a fragmentation sequence much like shown previously for sequence variants must be collected and processed.
- Routinely, the most abundant peptides are analyzed, whilst those >4 orders or lower can be missed.
- The authors deduced that much like peptides derived from the protein of interest will have unique elution times that those from HCPs would also have similarly unique times.
- Therefore, by combining a slightly higher load of material and a longer gradient, they would be able to pick out the HCP peptides in the space between the peptides from the API.

The White Space

- Much like SV analysis, the areas in the LC trace between the product peaks can contain a wealth of information.
- The instrument is continually collecting data and interrogating this can provide information.
- The authors were able to identify unique peptides within this area that were not derived from the product.
- Comparing these sequences to CHO or murine databases help identify the proteins.



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Dynamic Range

- To determine the capability of the assy they had designed Sperry and co-workers spikes protein standards into a Pfizer Mab sample.
- A comparison of the known concentration of the spiked proteins and the detected concentration demonstrates the capability to detect peptides down to 1 ng/mg.
  - This is equivalent to 1 ppm.
- Additionally, data interrogation suggested that some peptides were detectable to 0.01 ppm.

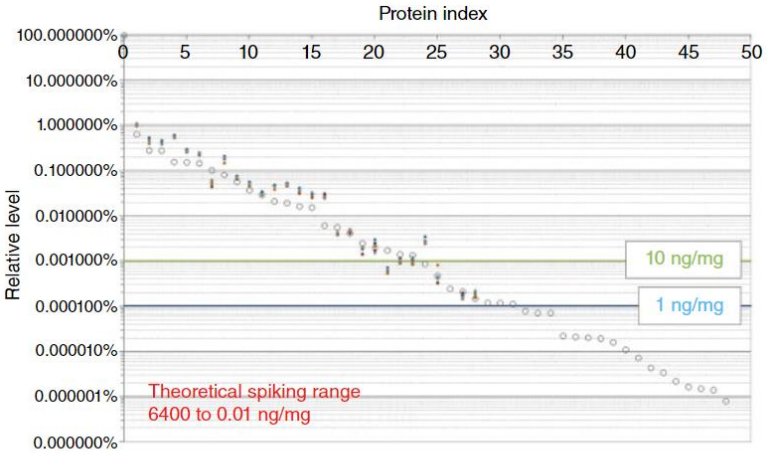

Figure 10.6 The detection of protein standards spiked in to mAb were definitiely achieved at 10 ng/mg and some at around 1 ng/mg.

Ref: Krawitz D. et al. (2017) Analytical Characterization of Biotherapeutics, 211-237

Case study – HCPs

- What is the advantage of knowing the HCPs?
- No two biologic cell line process is identical.
- The USP and DSP can enrich or lead to the co-purification of HCP proteins and there is no guarantee that the generic HCP kits can detect the prensence of your unique milieu.
- The method developed by Sperry and others provide a powerful orthogonal method when developing purity assays for the detection of resHCP.
- This data ensures that the specifications set for Ph 1 (i.e. NMT 100 ppm) are accurate and appropriate.

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Summary

- The development of analytical assays to support the progression of biologics into Ph 1 studies requires a deep understanding of the molecule.
- This can be guided by advice from regulators, the community or your prior experience.
- Maximizing the data collected during development and pre-clinical work helps guide not only assay feasibility but also appropriate specifications for later cGMP manufacture.
- For CDMOs, bringing the wealth of experience generated over numerous projects can help provide guidance and assurance to clients, especially those that may be entering this field for the first time.
- Here at CSIRO, we see ourselves in this role as trusted advisors.

Summary

- The development of a peptide mapping method is critical to not only providing assurance on the identity of the biologic
- But also, can generate data to help ensure that there are no aberrant post translational modifications present, which can include amino acid mutations
- Not all additional peaks in a peptide map can be assigned to the product.
- Sometimes these maybe resHCPs.
- The identification of these HCPs is critical to informing the safety release specifications and identifying improvements to process.

Thank you for your attention

Any Questions?

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