Supporting development and regulatory approval of biologics

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Introduction

To progress to later stage clinical trials, companies have to characterise their drug substance following ICH Q6B guidelines, profile process and product related impurities including those derived from contact and pathway materials, and ensure consistency of the raw materials used during manufacturing. RSSL undertakes ICH Q6B biologic characterisation and also uses similar techniques to test raw, contact and pathway materials. This poster focuses on representative examples of methods used during the profiling of process and product related impurities and testing of raw materials of biological origin.

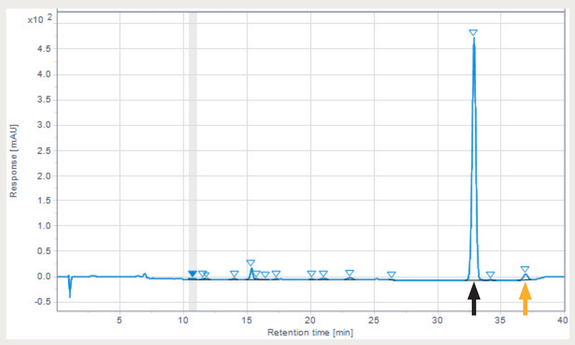
With the growth of biologics in advanced therapy medicinal products (ATMPs), additional raw materials including enzymes, lipid and viral vectors are becoming more common and require novel methods to be developed.

Product and process related impurities

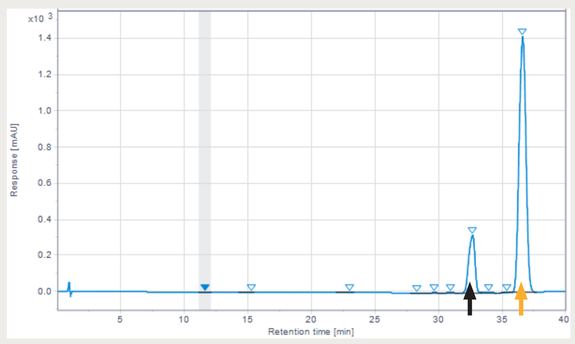
Manufacturing of the biologic drug substance can generate process related or product related impurities, that are not fully removed during downstream processing. Therefore, it is important to ensure all potential impurities are identified and limits defined (see Table 1) and analytical methods implemented to ensure that the manufacturing process and product quality is suitably controlled.

Impurity	Process or Product Related	Method that could be used
Aggregates	Process	Sub-visible particles, SEC, DLS
Degradation products	Process	HPLC methods (see Figure 1), cIEF, cSDS, WB
Host Cell Proteins	Product	ELISA, LC-MS/MS
Host Cell DNA	Product	qPCR (see Figure 2)
Vector-derived DNA	Product	qPCR

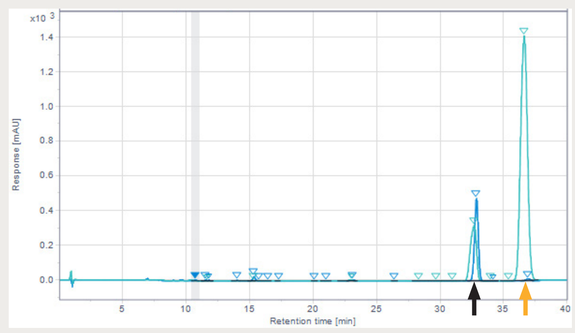
Table 1: Methods to detect impurities.



a) shows chromatogram of therapeutic (black arrow) and target degradant (yellow arrow).



b) collected isolate of 2nd large peak showing a small amount of therapeutic collected, but largely the impurity, which was freeze dried to concentrate.



c) Overlay of chromatograms showing purified impurity (b) relative to initial concentration of impurity (a).

Figure 1: Representative data showing PREPLC isolation and purification of a degradation related impurity.

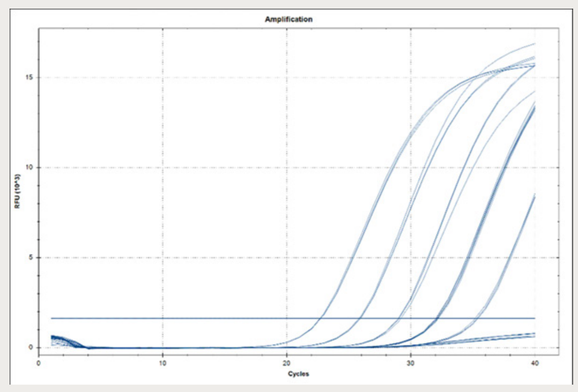


Figure 2: Representative example of qPCR instrument data showing the cQ values of standards being run in a host cell DNA assay.

Raw Materials (RM) testing

The overall aims to test raw materials (RM) are: a) identify the critical quality attributes, b) harmonize variable practices and make the regulatory expectations more predictable, c) encourage RM manufacturers to provide consistent, predefined quality, and to record and share information on the origin and quality of the RM, and d) help users manage batch-to-batch variations and changes.

For biological products, the testing of RMs may involve using established pharmacopeial methods, but typically there is a need to develop and validate novel methods designed specifically for the RM. As biologics move into ATMP, the development of assays for characterisation of enzymes, lipids and viral vectors is also critical.

This may include, but is not limited to: Appearance, Solubility, Osmolality, pH, Elemental impurities, Total protein, Related substances, Microbiological control, Viral contaminants, Bacterial endotoxins, Mycoplasma, Stabiliser, and Water.

RMs are tested to the required standards to ensure they are safe for human use. Where RMs are of biological origin, and no specific monograph exists, the General Chapter 5.2.12 of the EP may be relevant. The general requirements refer to: Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Material batch), Storage, and Labelling.

In terms of novel methods, there are many possible tests that can be performed to define RM characteristics that are important to ensure product consistency and to define the quality attributes of the raw materials. These tests must meet pre-defined quality requirements for identity, purity and biological activity. Although specific to drug substance and drug product, the new ICH Q14 guideline, designed to complement ICH Q2, provides for approaches that can be taken to design analytical methods and to define quality requirements for a method before analytical development starts. The methods must give consistent performance and undergo a validation process, in accordance with ICH Q2 guidelines before being used for routine testing. Therefore, the supply of reference materials, where possible, or the evaluation of the stability of representative batches of raw materials to ensure that the test is in control, must form part of any testing plan.

In terms of biological raw materials, examples of experimental approaches can be found in table 2. As well as developing novel methods from scratch, commercial kits designed to detect common analytes are available and can be used to create GMP validated raw material methods. Table 2 highlights techniques that can be performed within RSSL.

Sample Type	Testing Required	Suitable technique that can be implemented	Comments
Serum **Serum free cell culture media**	ID	SDS-PAGE/Western Blot. CE-SDS for protein profiling can be implemented if available volume is restricted.	SDS-PAGE provides a protein profile that can be used to confirm serum type. See Figure 3. When combined with a species-specific western blot, both species and serum type can be suitably identified.
	ID and/or Quantitative	ELISA	Confirm ID of component by a positive/negative result. A fully quantitative ELISA can confirm ID and concentration of component.
	Functionality	Cell bioassay Occasionally a bespoke ELISA	Activity of a cytokine can be tested with a customised bioassay. See Figure 4. e.g. for certain components, a bespoke ELISA could demonstrate depletion of specific cells by a RM – more cost effective than a cell-based method.
Defined protein components	ID and Characterisation	HPLC – Mass spectrometry	Intact mass – ID test for purified or expressed proteins. See Figure 5. Protein sequencing following tryptic and/or chymotryptic digestion. See Table 3. Identification of post-translational/in-process/storage modifications.
		HPLC – Protein	Purity – Reverse Phase or SEC. Aggregates/Oligomers – SEC, DLS.
		HPLC – N-linked Oligosaccharides, PNGaseF released	Fingerprinting/ sequencing to confirm correct glycosylation.
	Microbiological assays	Sterility, Bioburden and microbiological testing	Not required for all reagents but need to be considered when planning raw material testing.
Buffers	Microbiological assays	Sterility, Bioburden and microbiological testing	Not required for all reagents but need to be considered when planning raw material testing.

Table 2: Experimental techniques for biological raw materials.

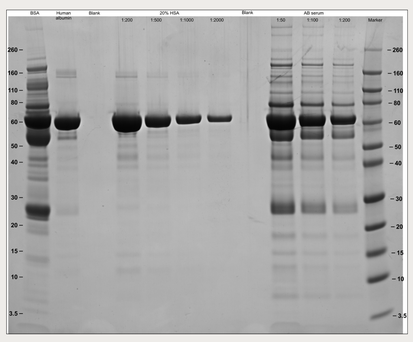


Figure 3: Human Serum Albumin versus Whole Human AB serum.

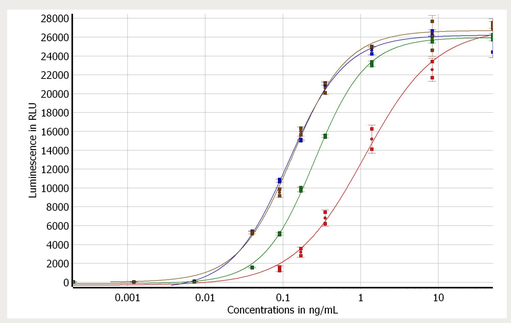
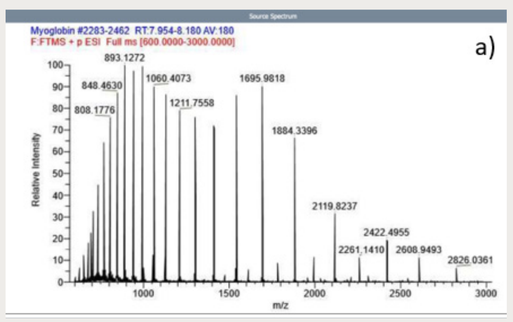


Figure 4: Recombinant cytokine at 100% (brown, blue) and 50% (green) versus EP standard (red line).



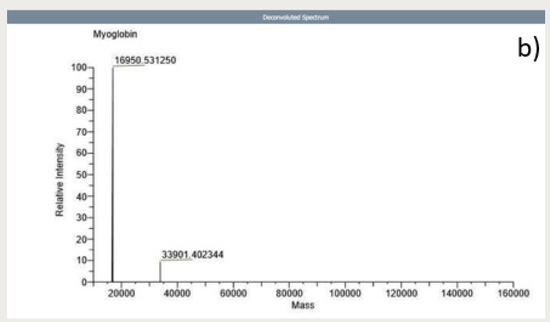


Figure 5: Representative example of an intact mass showing a) the source spectra and b) deconvoluted masses of a myoglobin system suitability control.

Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
367460260	Chain A, crystal structure of bovine serum albumin	14001.93	95.37%	175	3	99	920	583	66.4	5.86
74267962	ALB protein [Bos taurus]	12016.23	90.94%	151	4	91	836	607	69.2	6.25
229552	Albumin	11334.51	85.89%	134	2	87	792	581	66.1	6.09

Table 3: Representative example of a peptide map on a Bovine Serum Albumin sample.

Contact and Pathway materials

In addition to raw materials, contact and pathway materials also need to be tested – for example plastic tubing, bags, syringes, and other materials used to manufacture the final product of the biologic.

Material	Contact or Pathway related	Method that could be used
Syringes	Contact	Extractable & Leachable testing Sub-visible particle testing Sterility
Tubing	Contact & Pathway	
Media bags	Contact & Pathway	
Rubber stoppers	Contact	
Vials	Contact	

Table 4: Methods for single use materials in biologic final product manufacture.

Continuous monitoring of Raw, Contact and Pathway materials

Incoming testing of critical materials should be performed on a batch-to-batch basis. As part of incoming testing, continuous monitoring for foreign bodies (Figure 6 portrays a representative example), changes in supply, and sterility of RMs should also be performed.

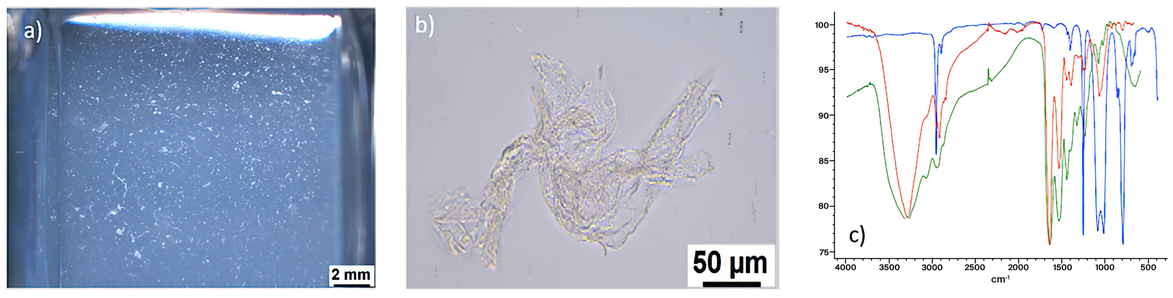


Figure 6: The steps of foreign body analysis that RSSL perform – starting with a) a visual check, followed by b) imaging with a light microscope and c) FTIR analysis to investigate foreign body identity (red line = sample; green line = protein reference; blue line = silicone reference).

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