

Table 6.9 Quality Attributes for mAbs

Category	Quality Attribute	Methods
Physicochemical Characterization		
Primary structure	Amino acid sequence	Reduced RP-HPLC-ESI-MS peptide mapping, intact mass of the whole mAbs, HC, and LC by RP-HPLC-ESI-MS, Red. RP-HPLC-UV peptide mapping
HOS	Disulfide bridging	Non-reduced RP-HPLC-ESI-MS peptide mapping
	Free thiols	Ellman's assay
	Secondary and tertiary structures	CD, FTIR, HDX-MS, X-ray
	Thermodynamic stability	DSC
General charge heterogeneity and amino acid modifications	OK variant, acidic variants, basic variant, Gin-variant, Lys-variant, amidated proline	CEX digested/undigested Boronate affinity
	Glycation	RP-HPLC-UV/MS peptide mapping
Glycosylation	Oxidation/deamidation/C-terminal variants Galactosylation, sialylation, mannosylation, afucosylation, bisecting GlcNAc, NGNA, α -galactose, qualitative glycosylation pattern	Normal phase-HPLC-FL
Size heterogeneity	Monomer, low-molecular weight and high-molecular weight variants (aggregates)	SEC, asymmetrical flow FFF
	Heavy chain, light chain, aglycosylated HC, clipped variants	Reduced CE-SDS
	Monomer, low-molecular weight (e.g., half antibodies and HHL variant) and high-molecular weight variants	Non-reduced CE-SDS
	Subvisible particles	Light obscuration (Peru, $\geq 10 \mu\text{m}$)
	Visible particles	Visual inspection (Peru)
Functional Characterization		
Target and receptor binding	Fern binding	SPR
	Facer binding (Curia, Fc γ R1IIa, Fc γ R1IIb, Fc γ R1IIa(F158), Fc γ R1IIa(V158), Fc γ R1IIb)	SPR
Bioactivity	CD20 target binding	Cell-based binding assay
	CDC potency	Cell-based CDC assay
	ADCC potency	Cell-based ADCC assay
	Apoptosis	Cell-based apoptosis assay

Note: HHL, heavy-heavy light chain; SPR, surface plasmon resonance.

measured. Since this exercise is highly dependent on the specific molecule, given below is a classification criterion for CQAs for mAbs:

- **Critical:** High mannose-type glycans (carbohydrate structures) are often a critical quality attribute. This attribute reduces the amount of time the drug stays in the body. This attribute can also be important for how a biological medicine functions and treats a particular disease. To have similar efficacy, safety, and immunogenicity to a reference product, a biosimilar needs to match all critical quality attributes.