chains and two light chains linked by a total of 16 inter- or intramolecular disulfide bonds. The two heavy chains are linked by disulfide bonds, and each heavy chain is disulfide bonded to a light chain. IgGs include Fab and Fc regions: The Fab is responsible for binding to the antigen, while the Fc binds to Fcγ receptors, which regulate immune responses.

Glycosylation is a common PTM for IgG antibodies produced by mammalian cells such as CHO cells, which are frequently used for production. IgG1 molecules contain a single N-linked glycan at Asn297 in each of the two heavy chains. During the synthesis of N-glycans, multiple sugar moieties can be added to form different glycoforms, e.g., G0, G1, G2 afucosylated complex. Glycosylation plays an important role in CDC and ADCC functions through modulating the binding to the Fcγ receptor. Particular glycoforms may be necessary to achieve therapeutic efficacy. These glycoforms may be targeted by glycosylation engineering but may also be affected by cell culture conditions.

Glycan testing is a complex and comprehensive analytical exercise. All glycan patterns can be judged from 2D PAGE analysis and tested for monosaccharides and sialic acid content. The N-glycans and O-glycans require determination of the site of glycosylation and profiling by MALDI and HPLC that allows determination of the content of each glycan. The structural heterogeneity of a glycoprotein can be ascertained using IEF or 2D PAGE. This is because sialic acids will lead to a shift in the pI of the glycoprotein, which can be determined. The cores of N- and O-linked glycans are largely composed of neutral monosaccharide building blocks, joined together by a specific stereochemistry. The stoichiometry and the identity of these building blocks are quantitatively analyzed by using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). This allows analysis at both N- and O-linked glycans in a single experiment. Usually, N-linked glycans are attached to the protein backbone by an N-acetylglucosamine and O-linked glycans by an O-N-acetylgalactosamine. Other typical neutral monosaccharides involved in N-linked glycosylation and O-linked glycosylation are fucose, galactose, and mannose. More specific testings may include analysis of monosaccharides released by acidic hydrolysis with HPAEC-PAD, investigation of intact N- or O-linked glycans by HPAEC-PAD, LC-ESI-MS or MALDI-MS, or visualization of the structural heterogeneity of a glycoprotein using 2D PAGE or intact mass.

Sialic acids are negatively charged, terminal monosaccharides and are important structural constituents of many glycans. The number of sialic acid molecules present impacts the activity and the serum stability of many glycoproteins. As different types of sialic acid can be incorporated into the glycan structure, it is important to be able to determine the presence of correct versus undesired sialic acid structures. Once sialic acids are released by acidic hydrolysis, it can be analyzed by HPAEC-PAD; also, N- and O-linked glycans carrying sialic acids can be tested by using HPAEC-PAD, LC-ESI-MS, or MALDI-MS.