

Demonstrating Consistent Antibody Glycosylation During Bioprocessing

a report by

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Dr Daryl L Fernandes set up Ludger in 1999. The company develops methods to measure and control biopharmaceutical glycosylation and provides a range of glycoprofiling systems and services to pharmaceutical and biotech companies worldwide. He was a consultant on biopharmaceutical glycoprofiling to Monsanto and GD Searle and helped spin out Oxford GlycoSciences (OGS) from the University. He joined OGS as Process Development Manager and was then Head of Analytical Services before founding Ludger. Dr Fernandes gained his doctorate at the Glycobiology Institute, University of Oxford.

Good control of product glycosylation during bioprocessing is of increasing importance to biomanufacturers of monoclonal antibodies (MAbs). There are four main reasons for this. Firstly, these biopharmaceuticals can show varying levels of non-human glycosylation and there are potential safety concerns. Secondly, glycosylation can significantly affect the efficacy profile of the therapeutic antibody by altering antigen binding characteristics and Fc effector functions as well as by modifying product stability *in vivo*. Thirdly, glycosylation can seriously impact on product consistency since changes in oligosaccharide patterns are a major cause of batch-to-batch variability during bioprocessing. Fourthly, antibody producers (both innovators and follow-on biologics companies) can gain commercial advantages by achieving good control and characterisation of the glycosylation of their therapeutic.

The concerns about antibody glycosylation are reflected by changes in the regulatory landscape, and authorities such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) are raising the standards that biomanufacturers must follow when demonstrating comparability of glycosylation for their biopharmaceutical production batches. The regulatory guidelines are now being harmonised under the International Conference on Harmonization (ICH) programme with the ICH Q6B guidelines covering glycan characterisation during therapeutic glycoprotein production and ICH Q5E guidelines dealing with characterisation after manufacturing changes.

This article outlines the main analytical profiles that can be used by biomanufacturers as part of an effective ICH-compliant system for measurement and control of MAb glycosylation.

Antibody Glycosylation

Therapeutic antibodies produced in mammalian expression systems bear two N-glycans in the CH₂ domain of the Fc region. This glycosylation

is highly heterogeneous and current MAbs typically contain up to 30 or so different types of glycans at each Fc N-glycan site. This 'micro-heterogeneity', together with the combinatorial pairing of glycans on the immunoglobulin (Ig)G heavy chains, leads to the presence of large numbers of different glycoforms in each product batch. The Fc glycosylation can significantly modify Fc effector functions, such as Fc receptor binding and complement activation. Furthermore, the effector function activities of different glycoforms can vary significantly and it is possible for a glycoform present in relatively low abundance to make a considerably large contribution to specific effector functions. Some MAbs (e.g. cetuximab, erbitux) also have Fab glycosylation, which can influence antigen binding affinity.

The MAb glycosylation pattern must be controlled during biopharmaceutical production to maintain the normal functional diversity of the therapeutic. The consequences of producing a MAb product batch with non-standard glycosylation can be serious. Safety issues include the production of potentially immunogenic glycoforms. For example, under certain conditions Chinese hamster ovaries (CHO) and murine cells can produce antibodies with oligosaccharides bearing N-glycolylneuraminic acid (NeuGc) residues or Gal α 1-3Gal disaccharide units (the Gallili antigen). These non-human glycans could produce immunogenic responses in patients. Furthermore, batches with aberrant glycosylation can exhibit non-standard efficacy profiles. For example, Genentech found that fucosylated glycoforms of herceptin showed a 40- to 50-fold decrease in the efficacy of Fc gamma-receptor (Fc γ R) III-mediated antibody-dependent cellular cytotoxicity (ADCC) compared with the non-fucosylated product. In this case, even a small variation in the fucosylated to non-fucosylated glycoforms could result in large changes to effector function profile of the antibody. The risk of such safety and efficacy issues can be significantly reduced by using a validated ICH-compliant glycoprofiling system to demonstrate consistent human-type glycosylation for each production batch.

Analyses for an ICH-compliant Antibody Glycoprofiling Scheme

Current licensed therapeutic antibodies typically display a set of over 30 different biantennary human-type N-glycans, which can be classified as follows:

- The number and type of acidic charged sialic acid residues (neutral [N], monosialylated [A1] or disialylated [A2] classes).
- The number of galactose residues (agalacto [G0], monogalacto [G1] or digalacto [G2] classes).
- The presence or absence of a bisecting N-acetylglucosamine residue between the two antennae of the glycan (\pm Bi classes).
- The presence or absence of core fucose (\pm F classes).
- Aberrant glycan structures.

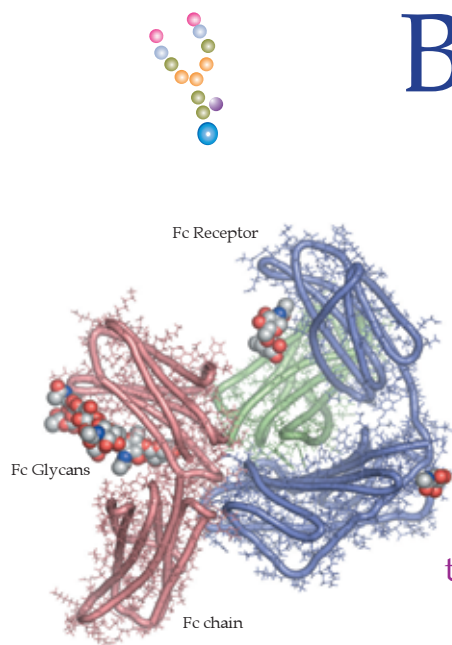
The product specifications for glycosylation of a monoclonal antibody relate to the desired pattern of these human-type oligosaccharides and the upper safe limits for the undesirable non-human or potentially immunogenic glycans. Biomanufacturers can demonstrate consistent, safe glycosylation patterns conforming to these specifications by measuring a range of glycosylation parameters, which include monosaccharide composition, oligosaccharide profile (for released glycans), glycosylation site profile (for

glycopeptides) and the glycoform profile (for the intact antibody). In practice, this can be achieved using a glyco-profiling scheme consisting of a subset of the following profiles, which are measured on either the intact glycoprotein or the released, fluorescently labelled glycans. These profiles are the ones most commonly used for quantitative glycoprofiling by the leading therapeutic antibody manufacturers.

Sialic Acid Profile

This indicates the relative levels of undesired non-human N-glycolyl-neuraminic acid (NeuGc) and desired human-type N-acetyl-neuraminic acid (NeuAc). The NeuAc level is related to the *in-vivo* clearance rate of the therapeutic. The sialic acid profile can be determined by releasing the sialyl residues from the glycoprotein by mild acid hydrolysis followed by fluorescent labelling of the released sialic acids with 1,2-diamino-4,5-methyleneoxybenzene (DMB) and then chromatographic profiling by fluorescence high performance liquid chromatography (HPLC) on a hydrophobic interaction column. ■

This article is continued, with graphics, in the Reference Section on the website supporting this briefing (www.touchbriefings.com).



Biopharmaceutical Glycosylation

Ludger specializes in measurement systems for biopharmaceutical glycosylation. Our glycan characterization technology is used by leading biomanufacturers worldwide.

For more information on our technology to measure and control glycosylation of your therapeutic glycoprotein and how you can comply with ICH Q6B and Q5E regulations contact us today at:

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