Analysis of Nivolumab, an IgG4 Subclass mAb, with Proteometer-L Assay

NOVILYTIC

BACKGROUND AND INTRODUCTION

Nivolumab is a monoclonal antibody (mAb) that is used to treat several cancers – and the list of approved indications is growing[1,2]. It is an IgG4 subclass of mAbs, which is the second most common type of therapeutic mAbs[3]. Nivolumab works as a checkpoint inhibitor and prevents cancer cells from evading recognition and clearance by the body's immune system. The reduced ability of IgG4 mAbs to interact with FcγRs, except FcγRI and FcγRIIb, is critical for their ability to function as immune checkpoint inhibitors[4].

Using Nivolumab as example, we demonstrate how to rapidly analyze the titer and relative aggregate content of IgG4 subclass mAbs in clarified fermentation broth (CFB) without the need for Protein A purification, using the Proteometer-L Kit. Monitoring changes in mAb concentration (titer) and aggregate content during the development and manufacture of a mAb is of critical importance as these fluctuations in quality attributes of the molecule can result in lower efficacy and/or higher toxicity of the drug. The use of the Proteometer-L Kit during product and process development for monitoring these important quality attributes affords time, labor, and monetary savings.

PRINCIPLE

Novilytic's Proteometer-L Kit uses the patented MASC™ technology to quantify mAbs without the need for purification from interfering matrix components such as host cell proteins present in fermentation broth. The kit uses a low molecular weight affinity selector to specifically detect mAbs through fluorescence coding, while contaminants remain invisible. The coding reaction is rapid and targets the Fc region of IgGs[5].

RESULTS

Nivolumab biosimilar (ichorbio, UK) was formulated at a concentration of 1 mg/mL in clarified fermentation broth (CFB) and injected in varying amounts onto an HPLC system equipped with a fluorescence detector. The Proteometer-L Kit was utilized for the system setup. The peak area responses from triplicate injections, performed on three different days (n = 9) were plotted against the amount (µg) of Nivolumab injected. The data showed excellent linearity from the range of 0.5 to 16 µg of mAb injected as shown in Figure 1 (R^2 = 0.9999). There is no interference from host cell proteins in the CFB sample matrix, and aggregate content is determined without the need for sample cleanup prior to analysis. Data from injections ≥ 4 µg level showed an average aggregate (high molecular weight mAb species) to total area percent of 0.61 ± 0.01 for 36 injections with coefficient of variance of 2.43%. Repeatability was evaluated by performing 10 consecutive injections of 4 µg Nivolumab in CFB in three assay runs, on three different days. Total area, representing the titer for the mAb showed excellent repeatability with an average area of 51,886,203 ± 606,922 for 30 injections and a CV of 1.17% (Figure 2). The aggregate percent was also consistent with an average of 0.60% ± 0.02 for 29 injections, and a CV of 4.04% (Figure 3).

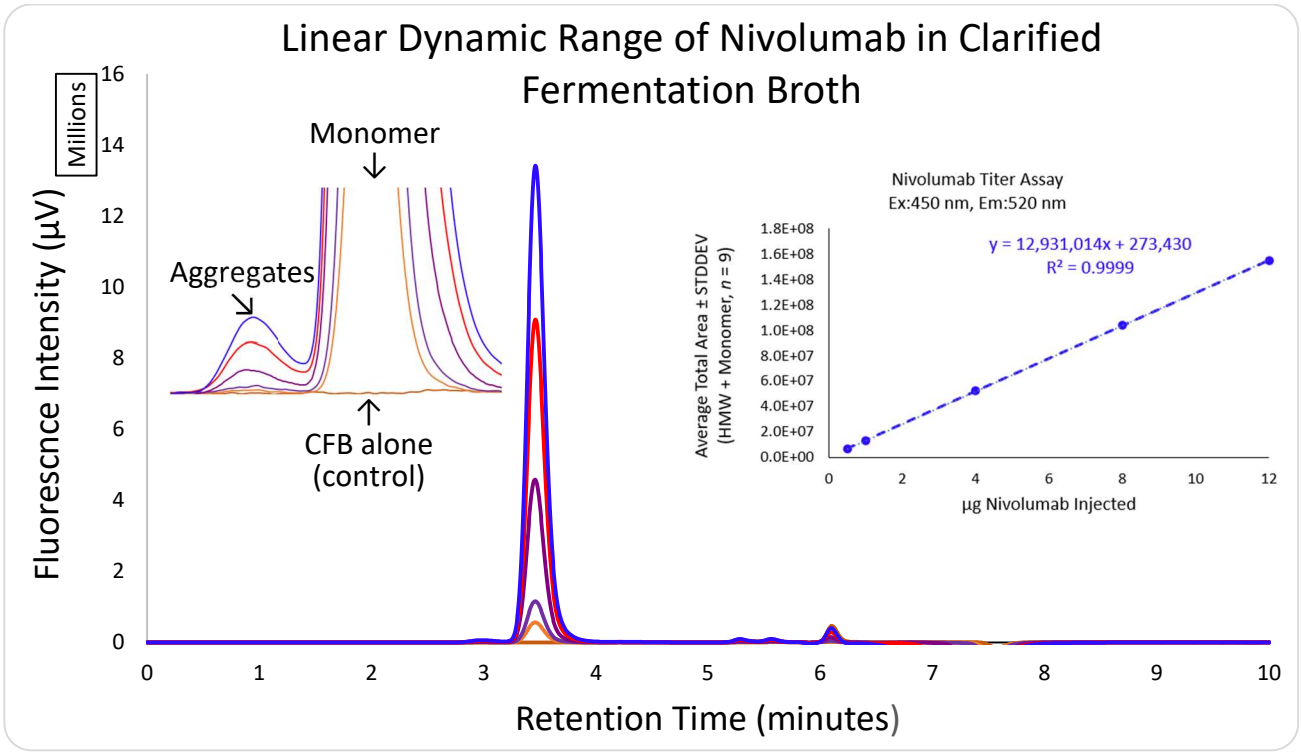

Fig. 1. Linear dynamic range of Nivolumab in CFB. Data is the average of three individual experiments with triplicate samples that were performed on different days using the Proteometer-L Assay.

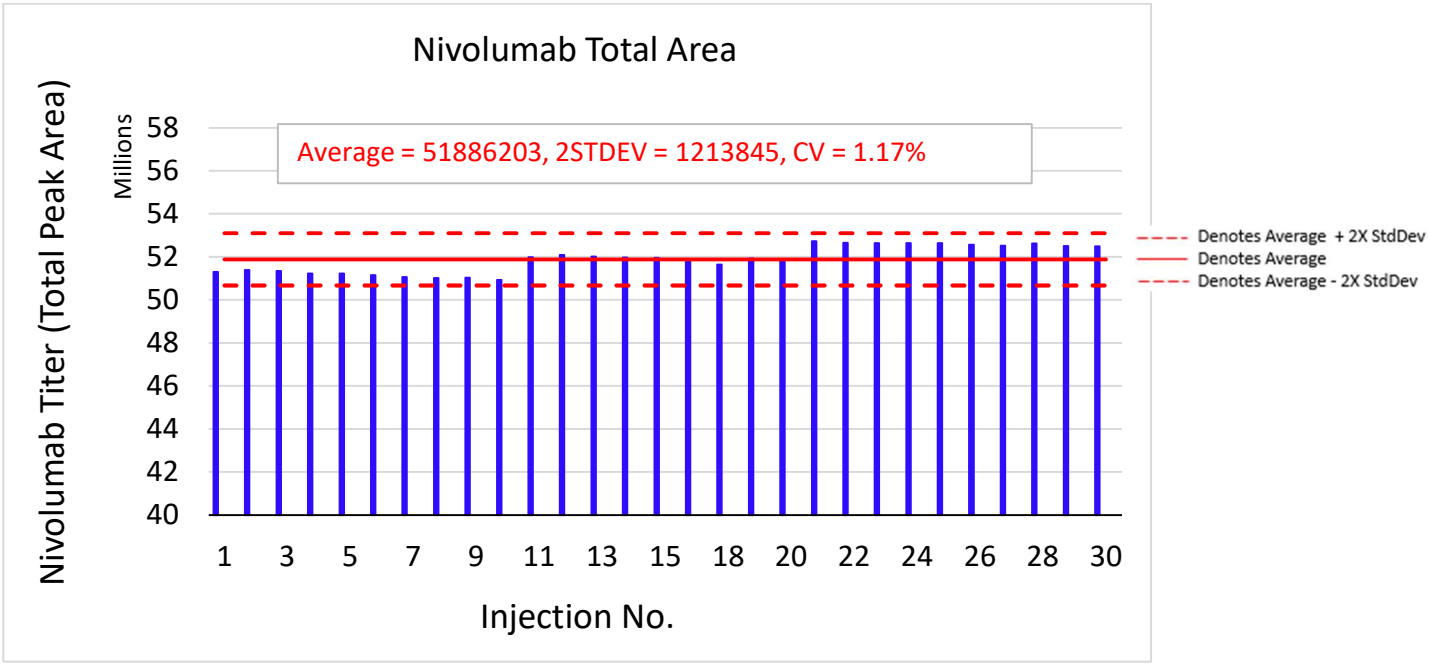

Fig. 2. Repeatability of total peak area observed in Nivolumab in CFB. Ten replicates of 4 µg injections were made on three different days for a total of 30 injections. Total area was calculated using integrated peak areas of aggregate and monomer species. Nivolumab titer (total peak area) is plotted against injection number.

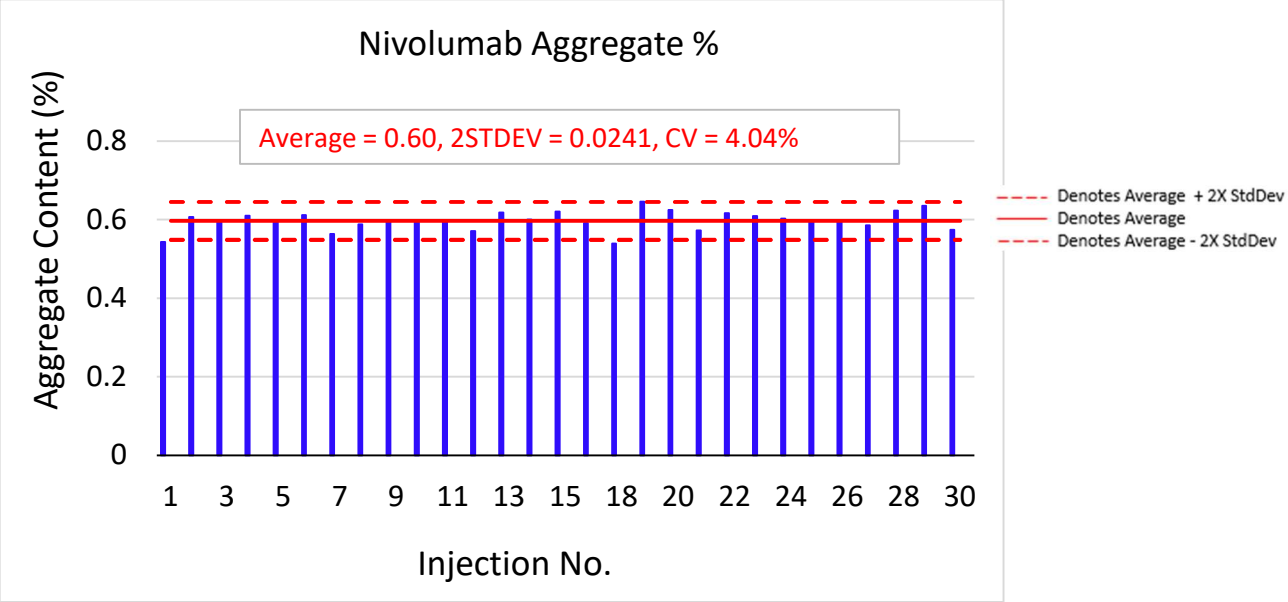

Fig. 3. Repeatability of aggregate content of Nivolumab in CFB. Ten replicates of 4 µg injections were made on three different days for a total of 30 injections. Percent aggregate was calculated using integrated peak areas of aggregate and monomer species.

REFERENCES

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