Titer Accuracy Comparison of Bispecific Antibody (bsAb) Using MASC Versus Protein A – UV

BACKGROUND AND INTRODUCTION

Typical antibody purification processes begin by harvesting bispecific antibodies (bsAbs) or monoclonal antibodies (mAbs) from bioreactors followed by membrane filtration. Purification and analyses steps follow, beginning with Protein A chromatography. This process can take up to 8 – 24 hours including sample preparation and Protein A purification, resulting in increased costs and changes in mAb structure with time and manipulation.

Product titer and aggregation is monitored at every step of product and process development. Using the new Mobile Affinity Selection Chromatography (MASC™) technology[1], Novilytic's Proteometer-L Kit provides mAb titer and relative aggregate content without costly and time-consuming Protein A purification. Samples are analyzed directly in clarified fermentation broth (CFB). The use of MASC technology also reduces the need for mass spectral analyses.

In this study, we compare the accuracy of bsAb titer results from Protein A purification and UV detection to titer results using Novilytic's Proteometer-L Kit.

PRINCIPLE

Size Exclusion Chromatography (SEC) is a widely used method for the analysis of aggregates in therapeutic bsAbs and mAbs (t-mAbs). Small aggregates like dimers, trimers, and tetramers, in addition to monomers and fragments are detected and quantified by monitoring UV absorbance at 280nm. mAbs in clarified fermentation broth (CFB) cannot be directly analyzed by this method, due to interference by proteins and metabolites at this wavelength.

Protein A affinity chromatography followed by UV detection is a commonly used LC-based method for mAb titer determination. This step in purification/quality testing slows the process down as well as adds accuracy concerns due to Protein A[2].

The Proteometer-L Kit utilizes a molecular recognition agent for the specific detection of antibodies by MASC thereby overcoming the time, costs, and inaccuracies associated with the Protein A methods. This new assay can be used to quantify proteoforms of intact human IgG molecules (IgG1, IgG2, and IgG4 subclasses). It is therefore suitable for the analyses of the vast majority of t-mAbs.

The Proteometer-L assay requires very small amounts of mAb, allowing for multiple analyses from 15 mL fermentors. The Proteometer-L assay offers enhanced sensitivity and has a wide and adjustable dynamic range of 1-80 µg mAb injected on column.

The Proteometer-L kit is an excellent replacement for the costly and time-consuming Protein A analysis currently favored by the industry.

EXPERIMENTAL

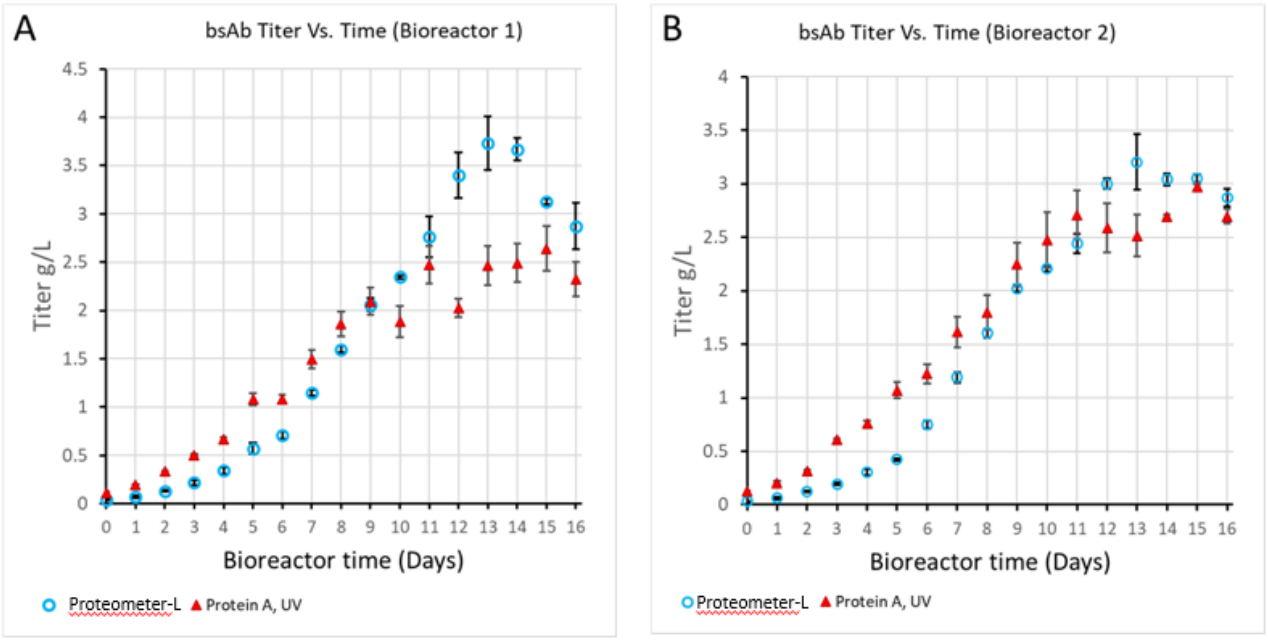

Fig. 1 Comparison of the temporal profiles of titer in the CFB of a therapeutic bispecific antibody (bsAb) with the Proteometer-L assay utilizing fluorescence detection, versus UV absorbance following Protein A purification. This data is courtesy of Johnson&Johnson[3].

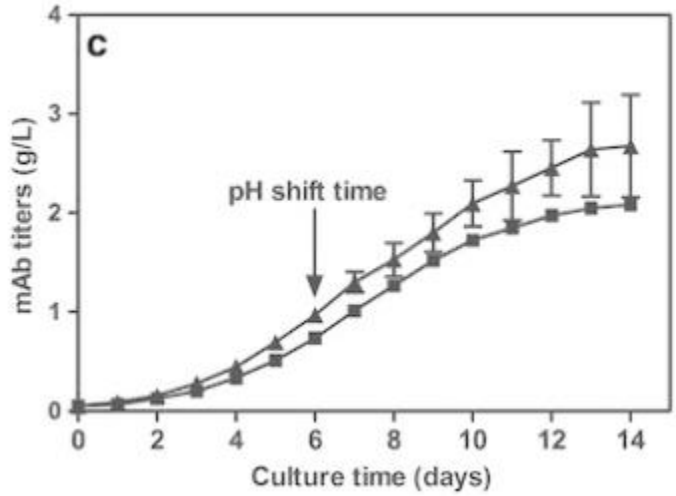

Fig. 2 Profiles of mAb titer during the fed-batch cultures with different culture pHs.

Notes and References:

1. Regnier, Fred, et. al.**,** Patent Application, "Molecular Recognition Assays of Critical Structure Attributes in Proteoforms", USPTO Application # 18/060,200, Ref# 2581-0011, Received 30 Nov 2022.

2. Dunn, Z.D., Desai, J., Leme, G.M., Stoll, D.R. and Richardson, D.D. (2020) Rapid two-dimensional Protein-A size exclusion chromatography of monoclonal antibodies for titer and aggregation measurements from harvested cell culture fluid samples. Mabs (Vol. 12, No. 1, p. 1702263).

3. Narsimhan ML, Kim J, Morris NA, Bower MA, Gunawardena HP, Bowen E, Regnier FE. Mobile Affinity Selection Chromatography Analysis of Therapeutic Monoclonal Antibodies. 2023. Analytical Chemistry, 95,16115-16122. Doi: 10.1021/acs.analchem.3c02180.