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

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. In production, further chromatographic polishing yields pure therapeutic products. This process takes 8 – 24 hours including sample preparation and Protein A purification.

Product titer and aggregation is monitored at every step of product and process development following a Protein A purification step. Using the new Mobile Affinity Sorbent Chromatography (MASC™) Luminon technology[1], Novilytic's Proteometer-L kit provides mAb titer and relative aggregate results while circumventing costly and time-consuming Protein A purification through direct analysis of crude culture filtrate (CFF). It is also important to note that the use of the MASC technology further reduces costly mass spectral analyses (when needed) by analyzing directly from the CFF.

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 the most widely used method for the assessment of size variants such as aggregates in therapeutic bsAbs and mAbs (t-mAbs). Small aggregates such as dimers, trimers, and tetramers, as well as monomers and fragments, are detected and quantified by monitoring the absorbance of the purified t-mAb analyte at 280nm. CFF may be directly analyzed by this method. It is well known that results may be inaccurate due to interference by proteins and metabolites at this wavelength. Also, additional processing time will be needed to integrate peaks which are not well separated from host-cell proteins.

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 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 (IgG1κ, IgG2κ, and IgG4κ subclasses). It is therefore suitable for the analyses of the vast majority of t-mAbs.

The assay is based on fluorescence detection of a conserved domain on IgGs by a molecular recognition agent that is proprietary to the MASC patent. The multitude of non-mAb CFF components are not detected, allowing mAbs to be analyzed directly from the fermentor. Both mAb titer and relative aggregate content can be determined in one run.

The Proteometer-L assay, unlike traditional methods, requires as little as 0.5 µg of antibody per assay, thus allowing multiple analyses even from 15 mL fermentors. Being dependent on fluorescence detection, the Proteometer L assay offers enhanced sensitivity and has a wide and adjustable dynamic range.

EXPERIMENTAL

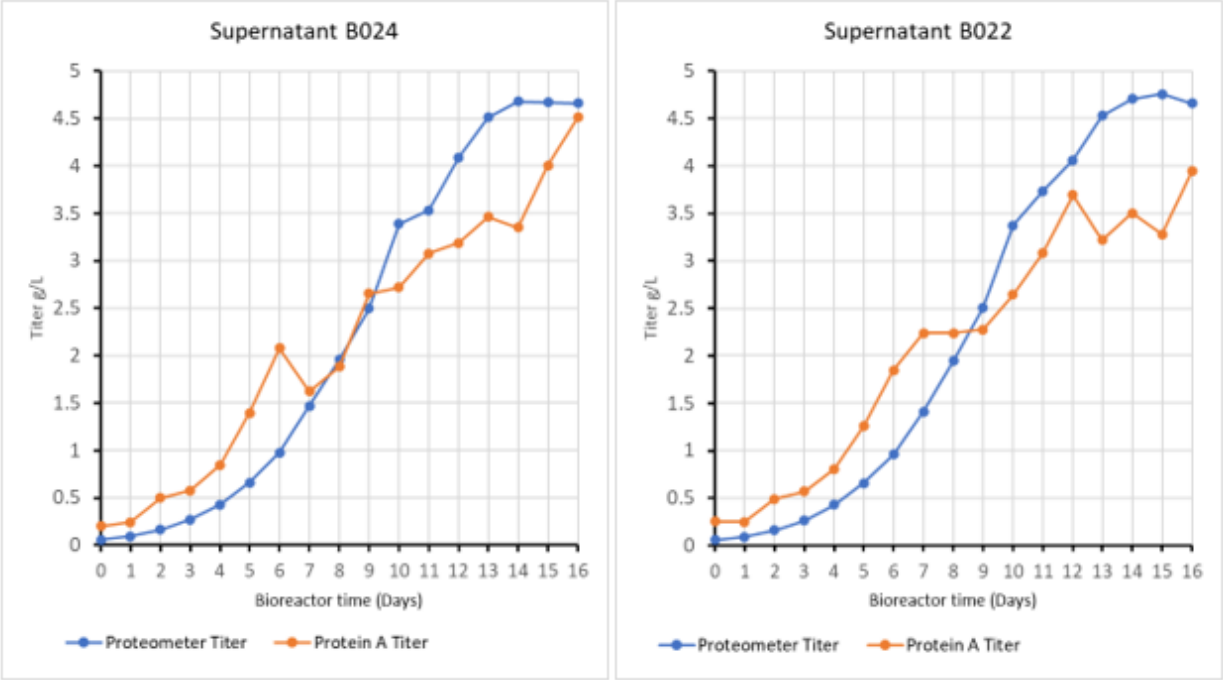

Fig. 1 Comparison of the temporal profiles of titer in the CFF of a therapeutic bispecific antibody (bsAb) with the Proteometer-L assay utilizing fluorescence detection, versus UV absorbance following Protein A purification. Test was completed over a 16-day period in two single-use 250mL reaction vessels of an Ambr 250 multi-parallel bioreactor system.

As seen in the figure above, the results obtained from the Proteometer-L kit track a cell growth curve closer and more consistently than the results obtained from Protein A followed by UV analysis. This shows the Proteometer-L kit to be an excellent replacement for the costly and time-consuming Protein A analysis currently favored by the industry.

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).