Identify Stable Biologic Candidates During Cell Line Development with Aura PTx

Introduction

Chinese hamster ovary (CHO) cell line development (CLD) has transformed modern therapeutic antibody production due to cell line's inherent scalability benefits, ability to produce proteins with the necessary mammalian post-translational modifications, large yield protein titers, and a longstanding regulatory record.[1] To fulfill the exponentially growing demand, CHO cell line developers have focused primarily on maximizing titer. However, newer and more complex biologic modalities like bispecifics and other types of antibodies that display low expression yield,[2,3] dramatically higher concentration requirements of injectables,[4] and large market demands[5] have pushed CHO cell lines to their limit. While progress has been made in titer characterization and ensuring biosimilarity, little has been done to characterize the physical stability of secreted antibodies from their inception during CLD. Instead, developability and formulation groups must stabilize biologics that were not designed with aggregation in mind from the ground up, often with limited success.

Aura™ systems are designed to characterize antibody stability as early as CLD. Aura+™ and Aura PTx™ deliver low volume, high-throughput subvisible particle imaging, counting, sizing, and identification. They easily handle and analyze biologically complex cellular and protein samples present in CLD to characterize secreted protein stability.

In this application note, we demonstrate how Aura PTx can quickly and accurately characterize secreted antibody stability during CHO CLD in unfiltered cell line samples. An established antibody labeling protocol (**Figure 1**) that requires as little as 40 µL per sample was used to characterize protein titer and rank cell lines according to the physical stability of the secreted antibody.

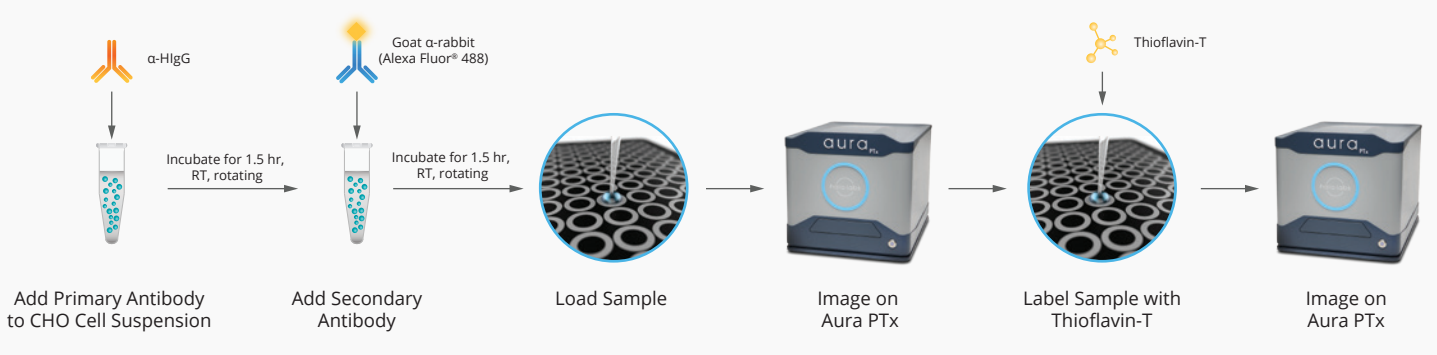

Figure 1: Schematic of the workflow using the Aura immunoassay to assess CHO stability during cell line development.

Material and Methods

Cell Lines

15 CHO cell line samples were obtained from a commercial partner. The 15 CHO samples were run in triplicate using 40 µL of sample per well.

Antibody Labeling

Cell suspensions were incubated with 1:200 α-human IgG (HIgG) primary antibody (Abcam #ab109489) for 1.5 hours at room temperature (RT) with rotation. The samples were then incubated with 1:100 goat α-rabbit IgG secondary antibody (AbCam #ab15007) conjugated to Alexa Fluor® 488 green fluorophore for 1.5 hours at RT.

Thioflavin T Preparation

5 mM Thioflavin-T (ThT) was resuspended in water for injection (WFI) by vortexing for 30 seconds and then filtered using a 0.2 µm syringe filter to remove any undissolved dye particles.

Aura Platform Analysis

Samples were imaged on Aura PTx using brightfield (BF), side illumination mode imaging (SIMI), and dual fluorescence. Fluorescence channel 1 (FL1) detected the protein aggregate specific dye, Thioflavin T (Ex 440/40, Em 500/40), and Fluorescence channel 2 (FL2) detected Alexa Fluor® 488 labeled particles (Ex 482/35, Em 524/24).

Results

Assessing Assay Specificity

Negative controls were first tested to ensure immunoassay specificity. Media only, primary antibody only, secondary antibody only, and primary plus secondary antibody samples (no epitope control) were stained with ThT, loaded onto a 96-well black membrane plate, and measured in all Aura PTx channels. The results show little to no background fluorescence, especially in the FL2 (green fluorophore) channel, confirming assay specificity.

Media was stained with ThT and incubated with both primary and secondary antibodies as described in the methods section, resulting in virtually no fluorescent signal from either fluorescent channel. The presence of small particles in the media was detected but not the antibody only controls, indicate that the particles are inherent to the media, not the antibodies (Figure 2a).

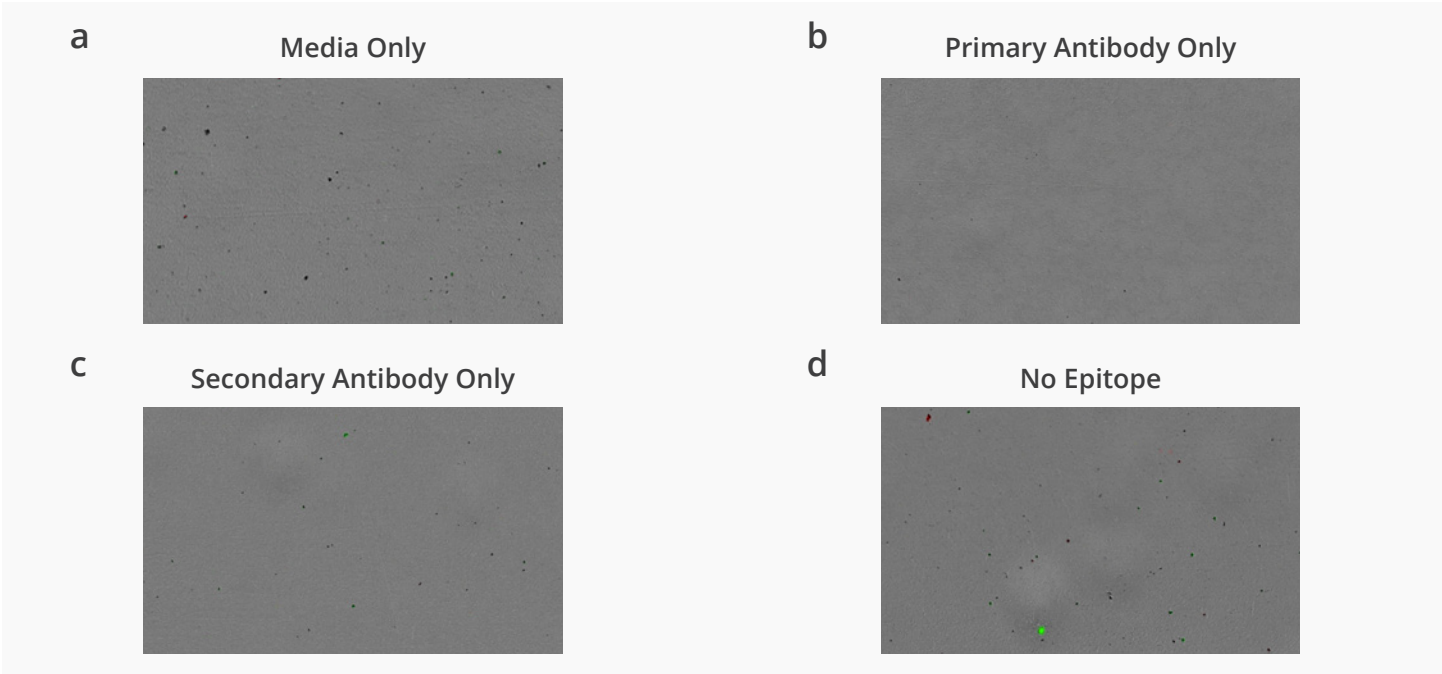

Figure 2: Negative controls confirm assay specificity. Low levels of fluorescence were observed in combined brightfield, FL1 (red), and FL2 (green) images for (a) media, (b) primary antibody, (c) secondary antibody, and (d) no epitope negative controls.

The primary antibody and secondary antibody controls were performed by incubating the respective antibody in PBS for 1.5 hours before loading onto the membrane. The primary antibody control sample displayed no fluorescent signal (Figure 2b) while the secondary antibody control displayed low level aggregation but displayed no fluorescent signal (Figure 2c).

Finally, a no epitope control that consisted of only primary and secondary antibody was assessed. A mixture of the antibodies were incubated in PBS for 1.5 hours before loading onto the membrane. This control also displayed low level of aggregation with minimal background signal in the FL2 channel (Figure 2d).

Cell Line Stability: Critical Insights using FMM

Various antibody secreting CHO cell lines were imaged and analyzed using Aura PTx. Figure 3 represents a range of samples with varying antibody stability from most stable (least aggregation) to least stable (most aggregation). Images were acquired in brightfield, FL1 ThT (red), and FL2 HIgG (green) channels. All figures represent the composite of all images acquired in all 3 channels. With only 40 µL of sample, these images allow us to quickly grasp the secreted antibody stability. In the data shown, Cell Line K was the most stable with almost zero antibody aggregation, Cell Line M was moderately stable, and Cell Line D was the least stable with several orders of aggregates above control observed.

The results demonstrate how Aura PTx can quickly visualize and easily differentiate between high-, mid-, and low-levels of antibody aggregation using the Aura immunoassay, and quickly distinguish if the particles measured are CHO cells or subvisible antibody aggregates.

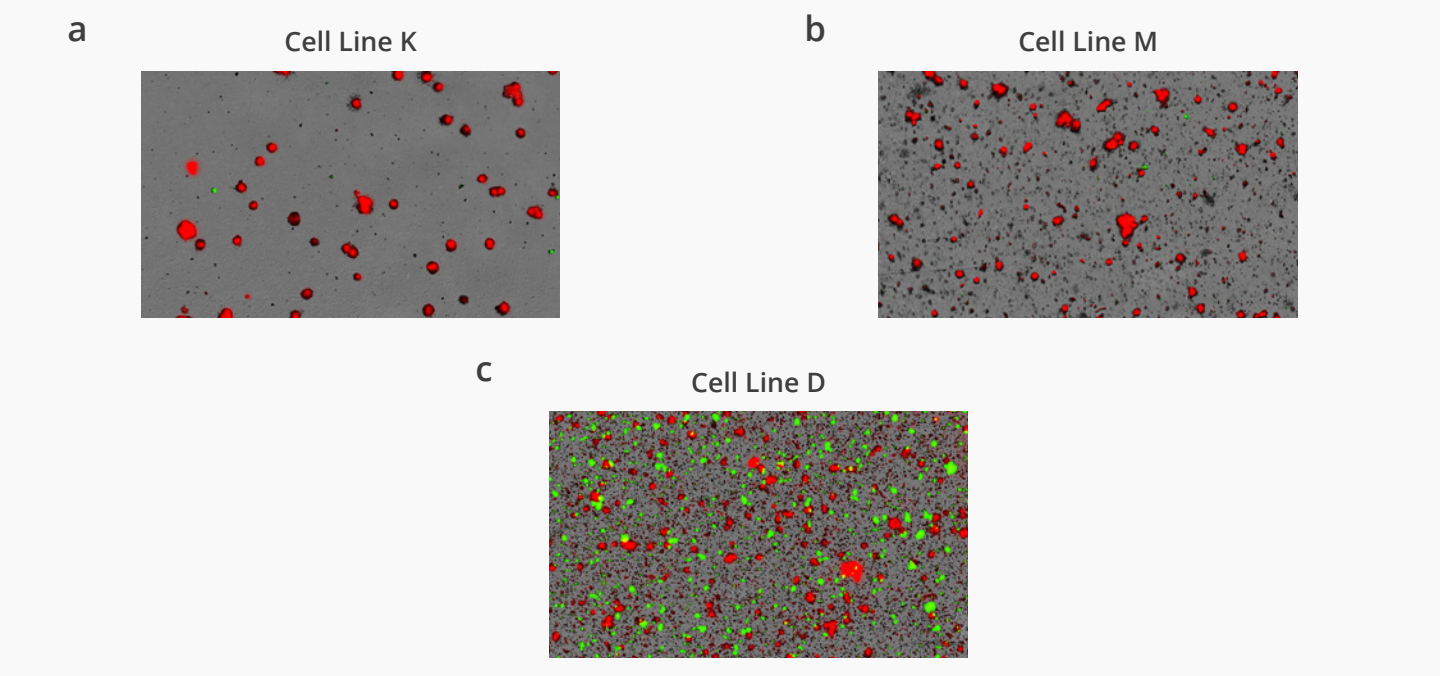

Figure 3: Stability analysis for antibody secreting CHO cell lines. Samples were imaged with brightfield, FL1 (ThT, red), and FL2 (HIgG, green) images on Aura PTx. (a) Minimal antibody aggregation was observed in Cell Line K. The low levels of aggregation observed was comparable to negative controls, indicating the inherent stability of this biologic candidate. (b) Cell Line M exhibited moderate to high levels of aggregation, indicating it is a moderately stable cell line. (c) Antibody aggregation several orders of magnitude higher than negative control samples were observed in Cell Line D, suggesting this biologic candidate could require more effort to develop a stable formulation.

Cell Line Rankings Based on mAb Stability

Aura PTx with Particle Vue software performs high-throughput characterization and identification of subvisible aggregates across an entire experiment, enabling the ranking of samples based on stability. The 15 cell line candidates were ranked for commercialization according to antibody stability (Table 1) based on the total area of particle fluorescence observed in the FL2 HIgG antibody channel (green). This area is a surrogate for the total mass of particles that fluoresced in the FL2 channel. A low FL2 area measurement (insignificant green signal across a sample) predicts a stable product since minimal subvisible antibody aggregates are formed. Conversely, a high FL2 area measurement (significant green signal across a sample) corresponds to an unstable product that produces large amounts of subvisible antibody aggregation.

Rank	Cell Line	FL2 Area (μm²)
	Primary only	885
	Secondary only	8,505
	Primary + Secondary (No Epitope)	14,826
	Media 1 (cells removed)	63,363
	Media 2 (no cells added)	63,442
1	K	67,198
2	O	88,691
3	J	95,815
4	N	127,443
5	M	148,400
6	L	163,991
7	B	343,631
8	F	908,819
9	A	1,298,386
10	I	1,442,221
11	C	2,564,835
12	E	4,213,704
13	G	4,516,363
14	H	4,770,222
15	D	5,045,953

Table 1: Cell line and control rankings by total level of HIgG positive cumulative area as measured by FMM. Cell lines colored light blue exhibited the lowest level of IgG labeling and are considered stable. Cell lines colored blue are moderately unstable, while cell lines colored orange are considered highly unstable. The FL2 area results were obtained using the Particle Vue software expression engine.

The immunoassay results display drastic differences between various CHO cell lines depending on the stability of the secreted antibodies. Specifically, Cell Lines K, O, and J displayed the lowest level of antibody aggregation, while Cell Line D displayed the highest level of aggregation. Cell Line N, M, L, B and F displayed minor aggregation, indicating these samples are likely easily stabilizable in formulation development and can be considered for commercialization along with Cell Lines K, O, and J. However, Cell Lines A, I, C, E, G, H, and D displayed dramatic aggregation, 12x to 50x above the negative controls, and could pose a significant challenge to stabilize.

Conclusions

Aura immunoassays bridge the gap between cell line development and developability to enable, for the first time, pre-screening for antibody stability once mAbs are secreted from CHO cells. Aura systems enable quick characterization of unfiltered cell lines samples through low-volume, high-throughput imaging, counting, sizing and identification. All analysis presented in this application note were performed with only 40 μL of sample per well (120 μL total across three replicates), and each well can be read in under two minutes. This enables painless analytical measurements that don't require entire flasks of cells.

Halo Labs' Particle Vue software automatically characterizes and quantifies the subvisible antibody aggregates visually and offers high-level ranking. The stored images deliver granular insights on every sample and particle to understand the types of aggregates that are formed. This gives you a deeper understanding of the physical instabilities of the biologic in question. In addition, Aura systems, powered by USP 1788 counting, enables stability tracking of a mAb from its inception through release, providing seamless method transfer.

References

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