

Impacts on Product Quality Attributes of Monoclonal Antibodies Produced in CHO Cell Bioreactor Cultures During Intentional Mycoplasma Contamination Events

Erica J. Fratz-Berilla¹, Phillip Angart¹, Ryan J. Graham^{2,3}, David N. Powers¹, Adil Mohammad³, Casey Kohnhorst⁴, Talia Faison¹, Sai Rashmika Velugula-Yellela¹, Nicholas Trunfio⁵, Cyrus Agarabi¹

¹U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Office of Pharmaceutical Quality, Office of Biotechnology Products, Division of Biotechnology Review and Research II, Silver Spring, MD.

²U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Office of Pharmaceutical Quality, Office of Testing and Research, Division of Product Quality Research, Silver Spring, MD.

³Currently with Genentech, San Francisco, CA ⁴Currently with Nova Biomedical, Waltham, MA ⁵Currently with Sartorius Stedim Data Analytics, Bohemia, NY.



Abstract

A mycoplasma contamination event in a biomanufacturing facility can result in costly cleanups and potential drug shortages. Mycoplasma may survive in mammalian cell cultures with only subtle changes to the culture and penetrate the standard 0.2 µm filters used in the clarification of harvested cell culture fluid. We studied the changes in the IgG1 product quality produced by CHO cells considered to be induced by the *M. arginini* contamination events. We observed changes in critical quality attributes correlated with the duration of contamination, including increased acidic charge variants and high mannose species. Also, partial least square (PLS) models using NIR spectral data were used to establish predictions of high levels ($\geq 10^4$ CFU/mL) of *M. arginini* contamination, but prediction of levels below 10⁴ CFU/mL were not reliable. Contamination of CHO cells with *M. arginini* resulted in significant reduction of antibody product quality, highlighting the importance of rapid microbiological testing and mycoplasma testing during particularly long upstream bioprocesses to ensure product safety and quality.

Introduction

Mycoplasma and Bioprocessing

- Mycoplasma are very small bacteria (0.1-0.3) µm that lack cell walls
 - They can penetrate 0.2 µm filters (pleomorphic)
 - If undetected, they could contaminate an entire production process
- Mycoplasma are occasional contaminants of cell cultures that produce biologics
- Mycoplasma can evade conventional bioburden assays

- In this report, we investigate the changes in IgG1 product quality produced by CHO cells induced by the *M. arginini* contamination events. We observed changes in charge variant profiles, purity, and glycan patterns that were further modeled using principal component analysis (PCA) to explore the relationships among *M. arginini* contamination, CHO cell growth and metabolites, and IgG1 product quality attributes.

Bioreactor Conditions

Briefly, we inoculated a recombinant CHO DG44 cell line that expresses a model chimeric IgG1 in a ReadyToProcess WAVE™ 25 rocker (GE, 2898800) operated in dual mode with two 2 L single-use cell bags (GE, CB0002L10-3) (1 L maximum operating volume) containing porous polyethylene-based perfusion filters. *M. arginini* was spiked into bioreactors either early in culture, prior to the start of perfusion when CHO cells were $\sim 2 \times 10^6$ cells/mL, or late in culture, during perfusion of 2 L/day when CHO cells were 9-12 $\times 10^6$ cells/mL and compared to control uncontaminated bioreactors. Control bioreactor cultures were confirmed to be uncontaminated during the entirety of the run. Each reactor was connected to a flow cell of a near-infrared (NIR) spectrometer (Sartorius Stedim, Göttingen, Germany) via the perfusion filter line in which culture material was continuously pumped either back into the bioreactor (batch mode) or into the perfusate collection carboy (perfusion mode). Three 14-19 day runs of two bioreactor cell bags, as described in Table 1, were completed.

Results and Discussion

Table 1. Description of bioreactor runs

| Bioreactor Name | Run Length (Days) | Day of mycoplasma spike | Mycoplasma spike level (CFU/mL) | Description |
|-----------------|-------------------|-------------------------|---------------------------------|--|
| Day 2-High | 14 | 2 | 220 | Model for early-stage high level bioreactor contamination |
| Day 2-Control | 14 | -- | -- | Control bioreactor (uncontaminated) |
| Day 3-High | 14 | 3 | 260 | Model for early-stage high level bioreactor contamination |
| Day 3-Control | 14 | -- | -- | Control bioreactor (uncontaminated) |
| Day 9-Low | 19 | 9 | 15 | Model for late-stage low level bioreactor contamination |
| Day 12-High | 19 | 12 | 300 | Model for late-stage high level bioreactor contamination and control for Day 9-Low bioreactor up to day 12 |

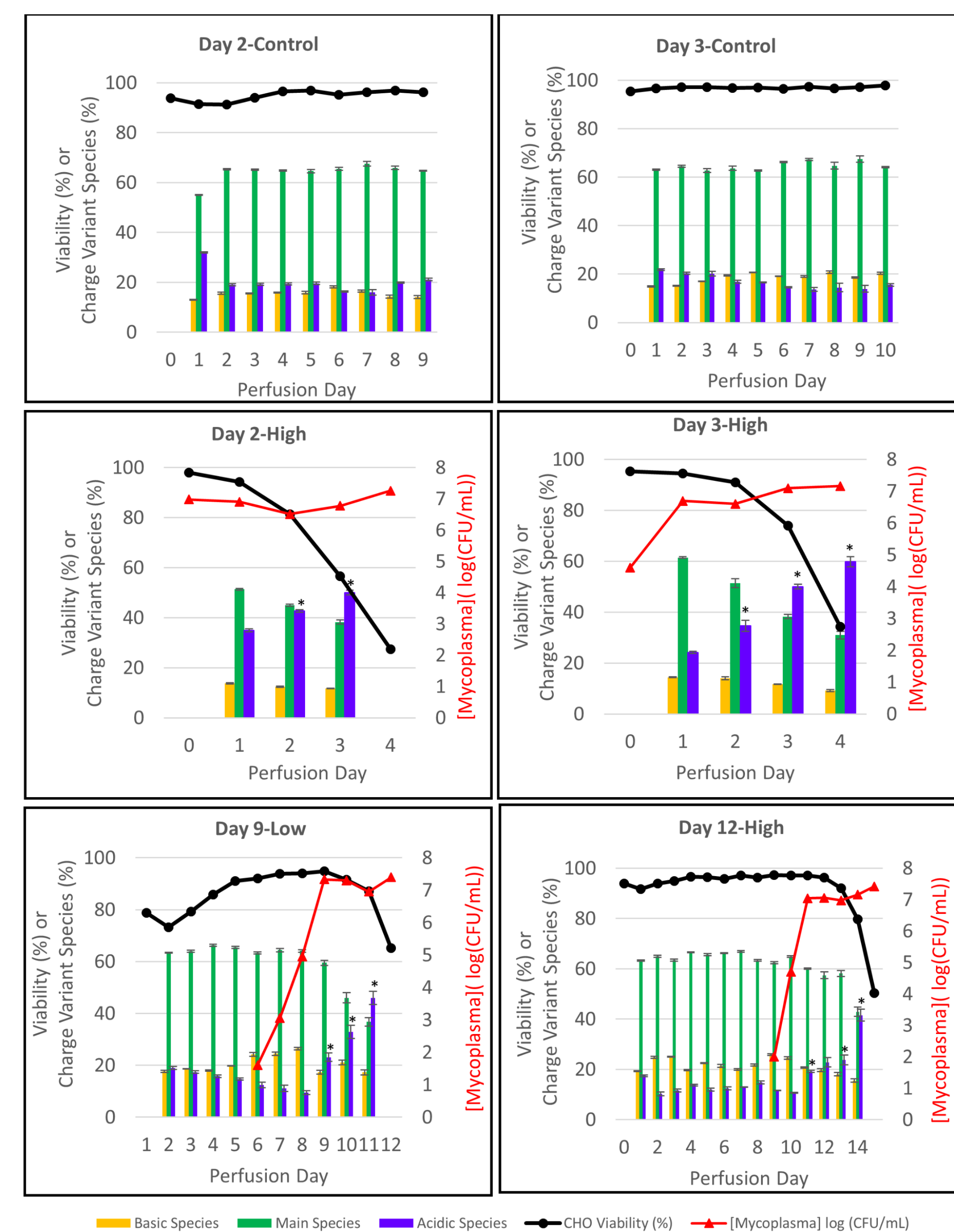


Figure 1. Charge distributions in mAb purified from control perfusion bioreactors and those contaminated with *M. arginini* by perfusion day.

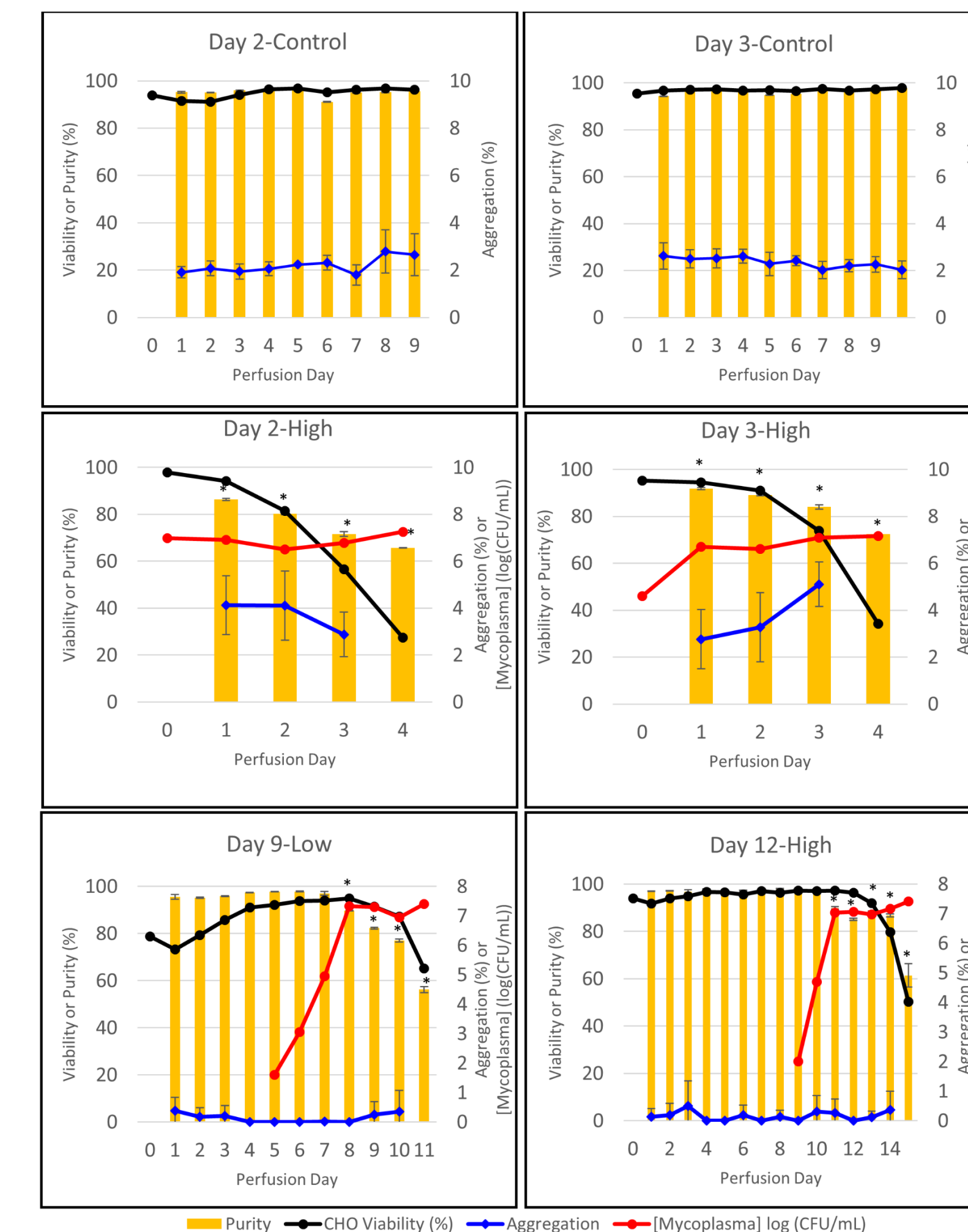


Figure 2. Purity and aggregation in mAb purified from control perfusion bioreactors and those contaminated with *M. arginini* by perfusion day.

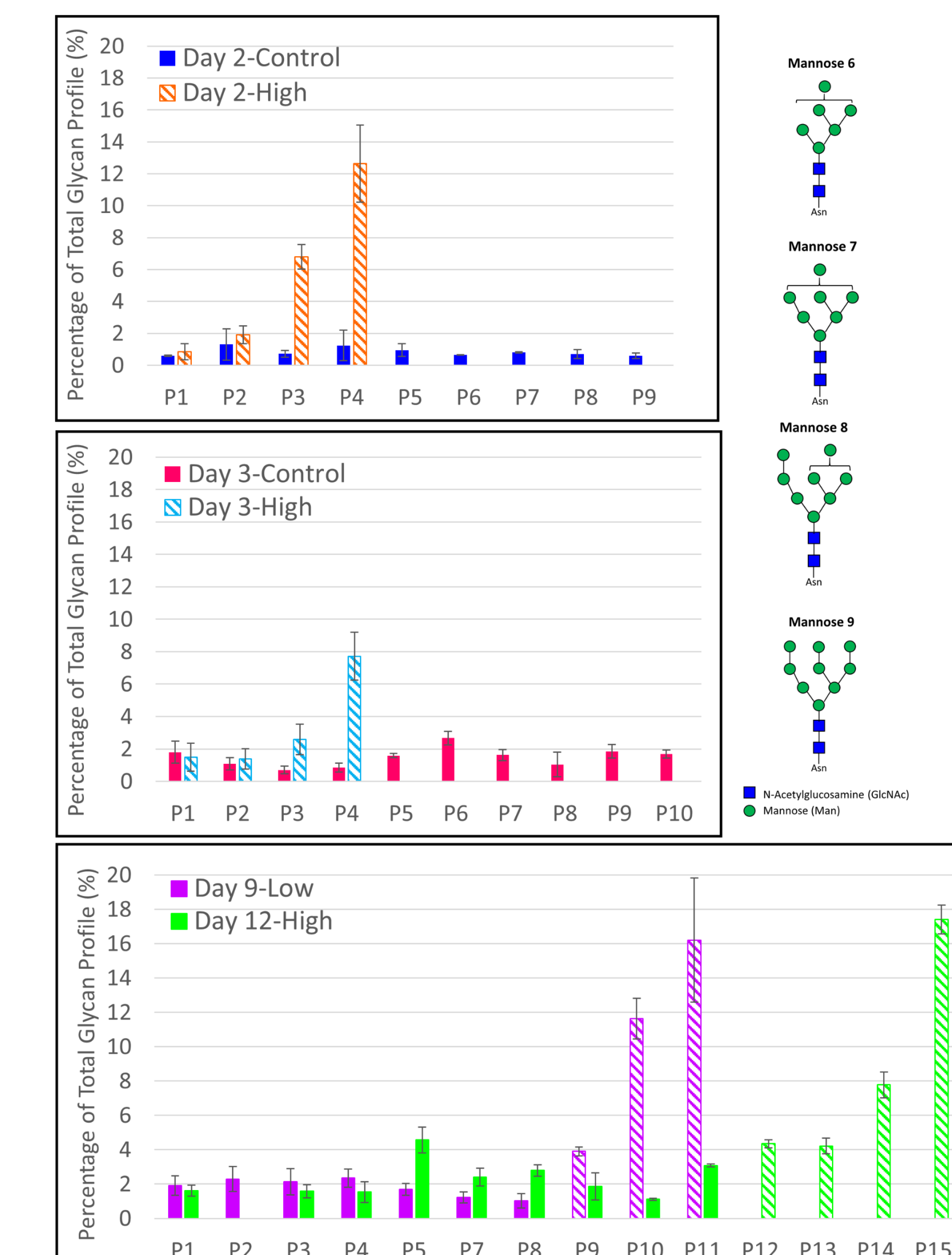


Figure 3. Mannose 6, 7, 8, and 9 species in mAb purified from control perfusion bioreactors and those contaminated with *M. arginini* by perfusion day.

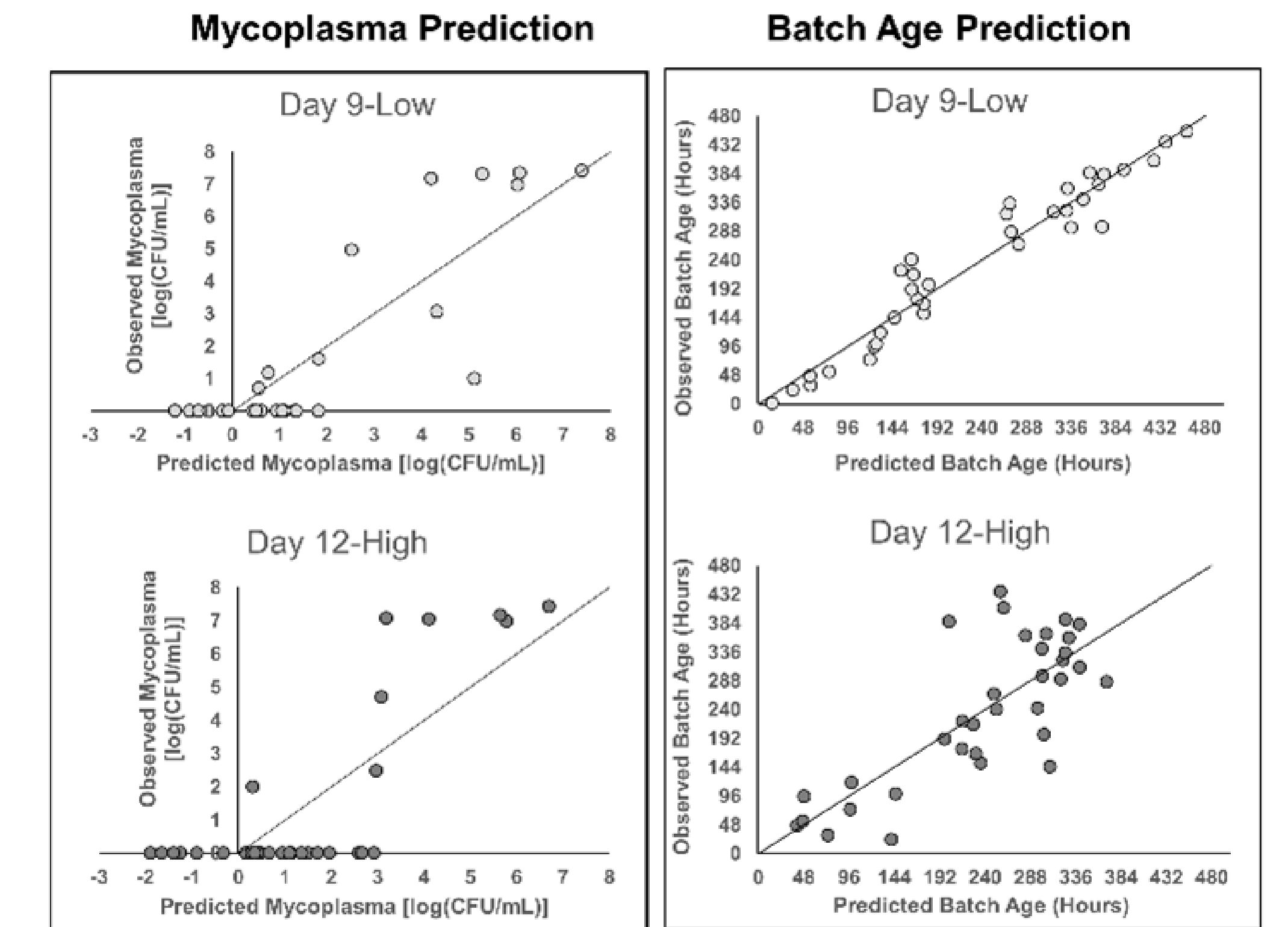


Figure 4. PLS models of NIR spectra to predicted mycoplasma contamination in Day 9-Low and Day 12-High.

NIR spectra can predict mycoplasma contaminations at high (4-8 log(CFU/mL)) concentrations, but some false positives occur at low mycoplasma concentrations. The batch age predictions indicate strong linear correlation and lack of polarized clustering between the predicted and observed batch age, which provides evidence of reliability of the saturated mycoplasma clusters in the prediction models.

Conclusion

- M. arginini* contamination resulted in the following mAb CQA effects:
 - Increased acidic charge variants
 - Decreased purity and increased DNA contamination
 - Increased high mannose species, especially those produced earliest in the oligosaccharide modification scheme
- Using PLS, we found that NIR spectra predicted mycoplasma contaminations at high (4-8 log(CFU/mL)) concentrations, but some false positives occur at low mycoplasma concentrations.
- With the growing desire to use spectral data, such as Raman and NIR, along with multivariate data analysis and modeling to monitor and control upstream bioprocesses, it may be important to understand what a contamination event, especially an otherwise occult contamination event, may have on bioprocess spectral data.

Please see our full publication of these data for further details: Fratz-Berilla EJ, Angart P, Graham RJ, et al. Impacts on product quality attributes of monoclonal antibodies produced in CHO cell bioreactor cultures during intentional mycoplasma contamination events. *Biotechnology and Bioengineering*. 2020;1-14.

Disclaimer

The opinions discussed in this poster are those of the authors and do not necessarily reflect FDA policy.