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Impact of raw materials on sialyation for a therapeutic protein

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Impact of Raw Materials on Sialyation for a Therapeutic Protein

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ABSTRACT

Total sialic acid content (TSAC) is a critical product quality attribute (CQA) for a therapeutic protein. By employing several statistical tools, we are able to identify certain lots of commercial culture media that cause differential levels of TSAC. Furthermore, we have identified several key components and their impact are evaluated in small scale production bioreactors. Overall this study provides the insight of understanding better control of product quality and establishes a methodology for identifying the root causes in cell culture media contributing to the variability of cell performance.

BACKGROUND

Total sialic acid content (TSAC) is a critical product quality attribute (CQA) for a therapeutic Protein manufactured by Alexion.

1. Sialic acid is a generic term for the *N*- or *O*-substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone.
2. The synthesis/modification is mainly distributed in endoplasmic reticulum or the Golgi apparatus (Figure 1)
3. It must be tightly controlled for the bulk drug substance (BDS).
4. Study results conducted by Alexion have suggested lot-to-lot variability of commercial cell culture media have potential effects on total sialic acid content (TSAC) at cell culture fluid (CCF) and harvested cell culture fluid (HCCF) steps.
5. Cell culture media, as used in this pipeline, is not chemically well defined, and is composed of wide array of unknown components.

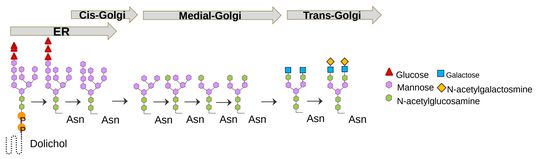

Figure 1: Schematic of glycosylation in cellular compartments.

METHODS

1. Employ multivariate analysis (MVA) to detect potential lots of raw material (RM) that cause differential level of TSAC.
2. Monitor differences of the cell performance by grouping raw material lots that result in differential levels of TSAC.
3. Measure levels of RM components such as trace metals, vitamins, and amino acids to be included in principle component analysis (PCA).
4. Employ PCA to classify RM lots and determine if ithey shares similar patterns of TSAC differences.
5. Evaluate the impact of top candidates suggested by PCA on TSAC in a small scale studies.

RESULTS

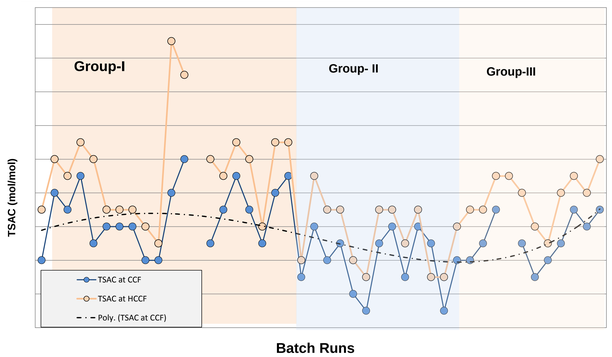

Figure 2: TSAC trending in manufacturing process

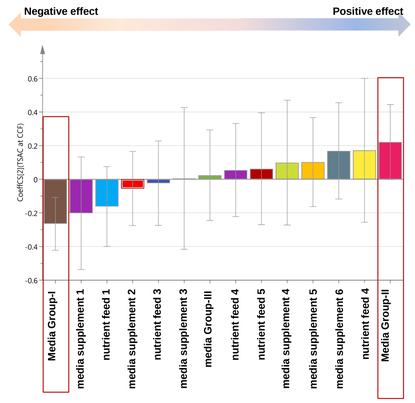

Figure 3: Impact evaluation of raw materials including media, media supplement, and nutrient feed on TSAC at CCF.

Batches using certain lots of media perform differently in TSAC level

1. Polynomial model suggests group II media lots result in low level of TSAC (Figure 2)
2. Group II media lots resulting in low TSAC were produced in Mar17- Apr17 (Table 1)
3. Multivariate analysis (MVA) suggests that only media groups have the highest and significant impact on TSAC, in comparison with media supplement and nutrient feed (Figure 3)
4. Using group II media lots in manufacturing scale results in statistically significant lower TSAC, compared to group I media lots (Figure 4)
5. Differential viability trending in cell performance is also observed. Batches using group II media lots demonstrate the lowest viability trending in comparison with other groups (Figure 5).

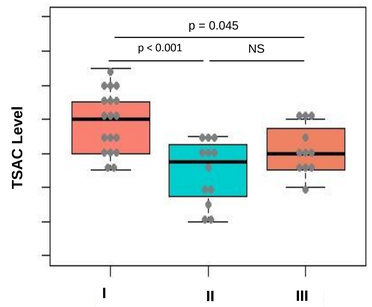

Figure 4: Comparison of distribution of TSAC at CCF from manufacturing cell culture using group I, II, or III of media.

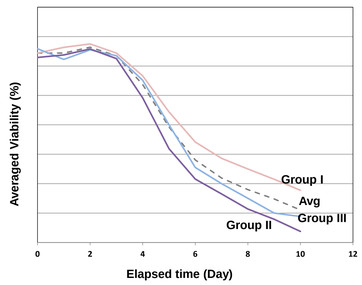

Figure 5: Comparison of viability trending from manufacturing cell culture using group I, II or III of media.

Analyzed Lot #	Group	Production time	Used in MFG	TSAC level in MFG
AB-1	I	2016		
AB-2	I	2016		
AB-3	I	2016		
AB-4	I	2016	√	
AB-5	I	2016	√	
AC-1	II	Mar17	√	
AC-2	II	Mar17	√	
AC-3	II	Mar17	√	
AC-4	III	Apr17	√	
AC-5	III	Apr17	√	
AC-6	III	Apr17	√	
AC-7	III	May17	√	
AC-8	III	Aug17	√	
AC-9	III	Aug17	√	
AC-10		Sep17		
AC-11		Nov17		
AC-12		Nov17		

	Low
	Medium low
	Expected

Table 1: overview of media lot production time and corresponding TSAC level.

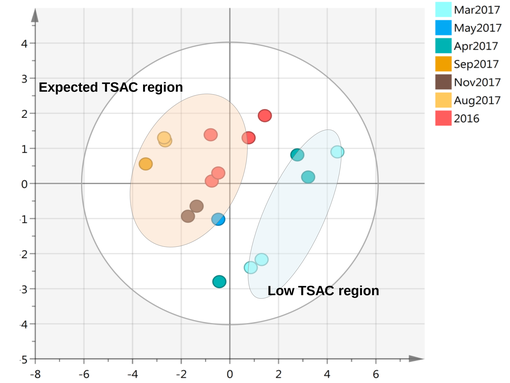

Figure 6 : Principle component analysis (PCA) classifies media lots.

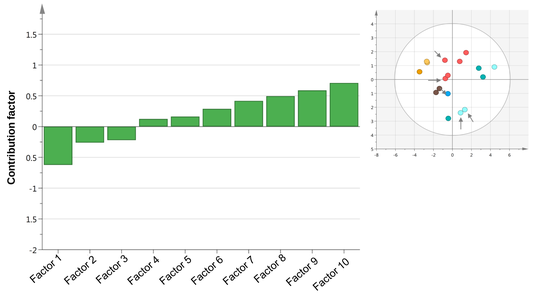

Figure 7: Contribution evaluation of media components by selectively analyzing media lots resulting in extremely high and low TSAC levels.

Principle Component Analysis (PCA) Classifies Media Lots in the Pattern of TSAC Levels.

1. Levels of trace metals, vitamins, and amino acids of media lots are included in principle component analysis (PCA).
2. PCA successfully clusters the media lots resulting in low TSAC (Figure 6).
3. Select media lots resulting in extremely high or low TSAC levels for contribution evaluation. The media lots with extreme TSAC levels are indicated by arrows (Figure 7).
4. Highly ranked factors are prioritized for TSAC evaluation in small scale (2L) production bioreactors.

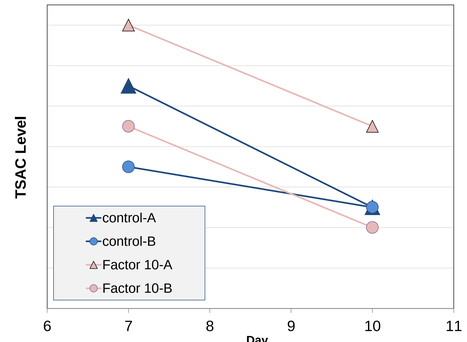

Figure 8: Day 7 and Day 10 TSAC results of duplicate spiking experiments in comparison with controls.

Top Candidate (factor 10) Spiking Experiment to Verify the Effect on TSAC by Using 2L Small-Scale-Bioreactors.

1. PCA and further analysis suggests that the level of factor 10 (Figure 8) could be positively correlated with TSAC level.
2. Day 7 results show that spiking results in high TSAC level in both duplicates
3. For day 10 results, one of the spiking duplicates shows higher TSAC level in comparison with its corresponding control, while the other demonstrates a similar level between spiking condition and control.
4. Factor 10 could be one of the root causes but its concentration effect needs further evaluation.

CONCLUSIONS

1. Raw material lot-to-lot variability could impact cell culture performance and TSAC levels.
2. Including levels of trace metals, vitamins, and amino acids in PCA allows us to identify manufacturing differences of media lots.
3. PCA provides a contribution ranking of each component for further evaluation.
4. Small-scale studies were performed and a potential root cause was identified .

FUTURE WORK

1. Continue evaluating top candidates determined by PCA or MVA in small scale studies.
2. Concentration and synergetic effects between validated factors should be carefully evaluated.
3. Provide feedback and establish collaboration with vendor to improve the consistency of RM production.

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Photo from left to right: Anne Kantardjieff, Krishanu Mathur, Jessica Kenney, Ina Alickolli, John Mavrianos, Bridget Leslie, Anjil Giri, Hunter Malanson, Jeremy Pike, Abraham Friedman, Daniel Kita, Patricia Bento, Lunedt Gonzalez, Natalia Adamson, Siguang Sui, Anthony Grecco, Loray Paul, Robert Ballinger, Mei Shao, Kyle Zingaro.