

## INVESTIGATING CELL CULTURE PROCESS PARAMETERS AND GALACTOSYLATION USING MEDIA BUFFERING EXPERIMENT AND DIGITAL TWIN MODELING

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Root cause analysis for out of trend cell culture processes can be a challenge due to confounding factors, multiparameter interactions, and missing data. Here, we present an example of large scale, cross-site variation of cell culture performance and galactosylation for mAb production. We identified that run-away, high CO<sub>2</sub> accumulation was due to the interference of three factors: process pH control, media buffering, and the lactate metabolism of cells. First, small-scale models were built to show the CO<sub>2</sub> and galactosylation correlation. The cause of sensitive CO<sub>2</sub> accumulation by pH control was confirmed by a medium buffering experiment. In parallel, in-silico cell culture models based on first principles were developed to predict CO<sub>2</sub> accumulation in terms of pH control, feed amounts, and lactate metabolism. The resultant process understanding, and methodologies of buffering experiments and digital twin modeling can be applied to manufacturing investigations and to a process control strategy to drive consistent cell culture performance and, at the same time, to reduce the number of small-scale lab studies.