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Advances in High-Throughput Upstream Process Development

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Author Bios

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Senior Director, Process Development, Cytovance Biologics

Co-Author of "The Effect of Temperature Shift on Product Titer and Viability in the Development of a CHO Platform"

Dr. Frank Agbogbo has over 16 years of experience in academia and industry. He is currently the senior director of process development at Cytovance Biologics and has been with the company since May 2014. He is currently responsible for leading the following R&D teams, cell line development, cell culture, strain development, fermentation, and midstream. Prior to Cytovance, Frank was the application technology manager at Mascoma Corporation where he contributed to the launch of two commercial products that are currently on the market. Prior to Mascoma, Frank was a principal scientist at ICM, Inc. and a scientist at Novozymes.

His background includes process development, Design of Experiments (DOE), process optimization, process characterization and scale-up from R&D to manufacturing (cGMP and non-cGMP). He was co-chair at scientific conferences such as the Society for Industrial Microbiology and Biotechnology Annual Meeting (SIMB) and Recent Advances in Fermentation Technology (RAFT) on many topics including process optimization, scale-up, DOE, process characterization, downstream development, and process analytical technologies. He has co-authored over 15 peer-reviewed publications and contributed to 5 patents. Frank holds a Bachelor of Science in Chemical Engineering from Kwame Nkrumah' University of Science and Technology, PhD in Chemical Engineering from Texas A&M University, College Station, TX and MBA with a certificate in Entrepreneurship from the Price College of Business, Oklahoma University, OK.

Sierra Bailey
Research Associate II, Cytovance Biologics

Co-Author of "The Effect of Temperature Shift on Product Titer and Viability in the Development of a CHO Platform"

Sierra Bailey has 4 years of industry experience. She is currently a Process Development Associate II at Cytovance, working primarily with mammalian cell culture. Her work at Cytovance includes running and managing process transfer and optimization experiments to ensure successful process transfer to Cytovance. She also writes process descriptions that are used to transfer processes from R&D to Manufacturing. Sierra holds an Associate's Degree of Diversified Studies of Science and a Certificate of Mastery in Biotechnology from Oklahoma City Community College.

Author Bios

Pawel Linke
Scientist Lead Identification, Roche

Co-Author of "Use Case: A Smart Path for Novel Biologics"

Pawel Linke has over 10 years of lab automation experience in industry. He is currently a scientist in Lead Identification at Roche Pharma Research and Early Development (pRED) and has been with the Roche since May 2017. He is responsible for developing end-to-end automated workflows including robotic systems with data engineering and analysis. Prior to this position, he was in the department of Cell Culture Research to implement automated sample analysis for the ambr® cell culture systems. His background includes assay development, process optimization, and data analysis.

From 2013 until 2017 Pawel was in Product Development for High-Throughput Systems at NanoTemper Technologies, and he was responsible for the development of the automated systems for measurement of MicroScale Thermophoresis (MST) and Nano differential scanning fluorimetry (nanoDSF). He contributed to the launch of two commercial products that are currently in the market. His prior experience was as a scientist for high-throughput screening at Pieris Pharmaceuticals. Pawel holds a Master of Science in Medical Biotechnology from Münster University of Applied Sciences.

In the present work, he implemented new screening process with increased throughput and flexibility including state of the art data analysis.

Markus Neubauer, PhD
Head of Bioprocess Research, Roche

Co-Author of "Use Case: A Smart Path for Novel Biologics"

Dr. Markus Neubauer was trained as a pharmacist and received his PhD in Pharmaceutical Technology in 2004. He has more than 16 years of experience in the medical device and pharmaceutical industry as a principal scientist and leader. He is currently the Head of Bioprocess Research in pharma Research and Early Development (pRED) at Roche. He is currently leading a R&D team that is responsible for cell line selection, upstream and downstream process development and supplies for early-stage technical development. Prior to Roche, Markus was a scientist and department head at the medical device company Gambro (now Baxter) working on development of medical devices and stem cell therapies. In his career, he has worked on various topics (medical device, consultancy, pharma) with various drug modalities ranging from (stem) cell technologies and therapies, exosomes, protein biotherapeutics to gene therapies.

His background includes pharmaceutical technology, medical device development, process development of biotherapeutics as well as stem cell technologies and therapies. He has co-authored 15 peer-reviewed publications and contributed to six granted patents. Markus holds a license as Pharmacist and a PhD in Pharmaceutical Technology from the University of Regensburg, Germany. He did a scholarship at Kyoto University, Kyoto, Japan in 2003.

Author Bios

Brandy Sargent
Editor-in-Chief, *Cell Culture Dish* & *Downstream Column*

Author of "Doing More with Less: Maximizing Phase Appropriate DOE Strategies - Keynote Summary**"**

Brandy Sargent is the Editor-in-Chief and frequent author of *The Cell Culture Dish* and *The Downstream Column*, She has worked in the biotechnology industry for over twenty years, first in corporate communications and public relations, then in technical sales and marketing, and most recently as a writer and publisher. She strives to introduce topics that are interesting, thought provoking, and possible starting points for discussion by the biomanufacturing community. She has been fascinated by the different applications of biotechnology since she first started working in the industry and continues to be fascinated as the industry evolves.

Patricia McNeill
Associate Director, Cell Culture Development, Lundbeck Seattle BioPharmaceuticals, Inc.

Interviewee in "Phase Appropriate DOE**"**

Patricia McNeill joined Lundbeck Seattle BioPharmaceuticals, Inc. in October 2019 as part of the acquisition of Alder Biopharmaceuticals. As Associate Director of Cell Culture Development, she has overseen the successful upstream process development for early to late stage cell culture and fermentation processes producing therapeutic monoclonal antibodies. She was hired as the 10th employee at Alder and has since held progressive leadership roles including the build-out and expansion of the research development lab for yeast fermentation. As Alder grew and new production platforms were needed, Patricia was tasked in establishing a CHO cell culture platform from the ground up. Prior to Alder, Patricia worked at Corixa Corporation where she was responsible for production of *E. coli* antigens used in cancer vaccine therapies. Her passion is using Design of Experiment (DOE) studies to decrease Cost of Goods (COGs), increase process efficiency, and robustness, and speed up development timelines.

Tim Noetzel, PhD
Manager of Lab Data Solutions, Roche

Author of "Organizational Setup to Integrate Data Management, Software Development, and User Convenience from Research to GMP Production in Pharma Biotech**"**

Dr. Tim Noetzel is currently Manager of Lab Data Solutions for Roche Pharma Manufacturing Science and Technology at Biotech Production in Penzberg, Germany. In this role he leads a team responsible for operating the Sm@rtLine Data Cockpit (SDC), the Laboratory Management and Information System (LIMS) as well as several equipment entities. Prior to his role in Lab IT, Tim was responsible for operating a large eukaryotic cell culture fermentation line as well as seed train cultivation, fermentation process analytics and served as responsible production process owner.

Tim partners with Operational Excellence, Quality Compliance, Pharma Research, and the Roche Pharma digital transformation team to improve efficiency, productivity and product quality. Tim holds a PhD in molecular biology from the Technical University of Dresden and has worked as a research scientist for the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden.

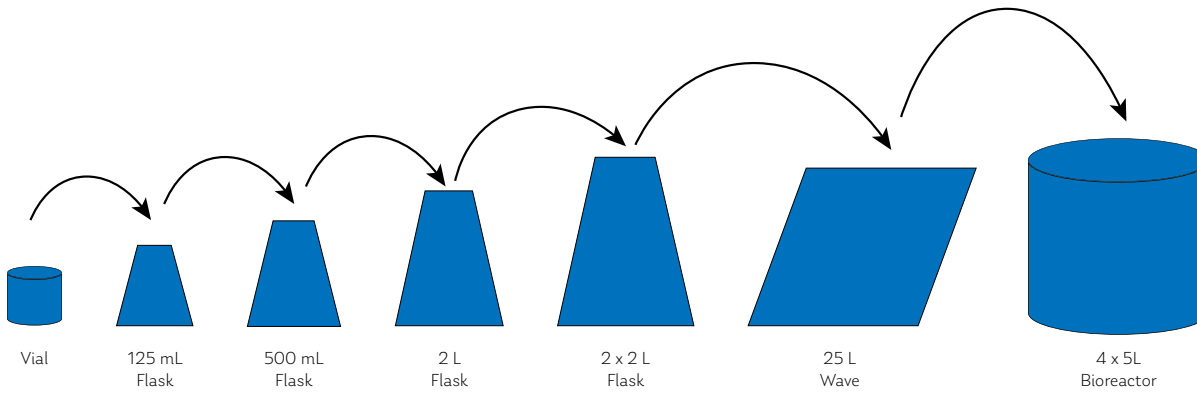
The Effect of Temperature Shift on Product Titer and Viability in the Development of a CHO Platform

Sierra Bailey and Frank Agbogbo

Developing a platform process to accelerate the development of other therapeutic proteins.

A MAMMALIAN EXPRESSION VECTOR ENCODING THE TRASTUZUMAB MONOCLONAL ANTIBODY WAS TRANSFECTED INTO THE CHO-K1 DERIVED CHOKC, AND A CLONAL CELL LINE WAS CULTURED IN A FED-BATCH CELL CULTURE PROCESS BY USING SHAKE FLASKS, A WAVE BIOREACTOR, AND 4 X 5L BIOREACTORS. THE VIABLE CELL DENSITIES FOR EACH SPLIT WERE MEASURED WITH THE VI-CELL XR BIOANALYZER FROM BECKMAN COULTER. THE GLUCOSE, LACTATE AND IgG TITER WERE MEASURED BY A CEDEX BIO ANALYZER FROM ROCHE CUSTOMBIOTECH. TWO CONDITIONS WERE TESTED IN THE 4 X 5L BIOREACTORS: 2 X 5L WITH NO TEMPERATURE SHIFT (37˚C THROUGHOUT) AND 2 X 5L WITH A TEMPERATURE SHIFT (STARTING AT 37˚C AND SHIFTING TO 32˚C ON DAY 6). THERE WAS A 76% IMPROVEMENT IN HARVEST PRODUCT TITER WHEN THE TEMPERATURE WAS MAINTAINED AT 37˚C THROUGHOUT THE BIOREACTOR COMPARED TO WHEN THERE WAS A TEMPERATURE SHIFT TO 32˚C.

FIGURE 1: Process flow for 5L bioreactor campaign.



Introduction

The production of recombinant therapeutic proteins requires the use of a host organism. The host organism that are commonly used for therapeutic proteins include CHO, *E. coli*, *P. Pastoris*, and *S. Cerevisiae* (1). The popularity of CHO for mammalian cell-based production is mainly due to its capacity for efficient post-translational modifications and ability to produce recombinant proteins with glycoforms compatible with humans at large manufacturing scales (2). CHO cells have been demonstrated as safe hosts for recombinant therapeutic proteins for over three decades (2). There are many therapeutic proteins approved by the FDA that are produced in various CHO cells such as CHO-S, CHO-K1, CHOK1SV, CHO DG44 (3). There is a lot of interest in decreasing time and effort required in process development of therapeutic products (4). One method that is used to decrease time and effort is the development of a platform process (5).

The CHOKC cell line was developed at Cytovance by using the adherent CHO-K1 cell line. The original CHO-K1 cell lines were serially adapted into animal component-free (ACF) commercially available media. These cells were used to make the CHOKC Research Cell Bank (RCB). The cells were transfected with the pD2534 vector (ATUM) engineered to produce the trastuzumab biosimilar. The stable clones from the transfection were scaled from shake flask to a 25 L wave bioreactor and finally into 4 x 5L bioreactors. The process for producing therapeutic proteins in CHO cells can be performed with or without a temperature shift during the cell culture. Thus, the CHOKC cell line was evaluated in 4 x 5L bioreactors with and without a temperature shift to determine the impact of a temperature shift on product titer and viability. The process steps used are shown in FIGURE 1. The results of the bioreactor campaign as well as decisions on glucose levels and product titer are discussed in this

work. Our understanding of the CHOKC trastuzumab process will enable the establishment of a platform process that can accelerate the development times for other therapeutic proteins.

Materials and Methods

One vial of the CHOKC Development Cell Bank (DCB) was thawed and cultured for six passages. The seed train was cultured in BalanCD CHO Growth A media containing 8 mM L-Glutamine and 1% v/v anti-clumping agent. The cells were seeded at a viable cell density (VCD) of 0.4 million cells/mL, and they were split when the VCD was greater or equal to 3 million cells/mL. During the 6th passage, the cells from the seed train were used to seed 4 x 5L bioreactors. The operating parameters for the bioreactors are shown in TABLE 1. All bioreactors were

TABLE 1: Operating parameters for the 5L fed-batch bioreactors.

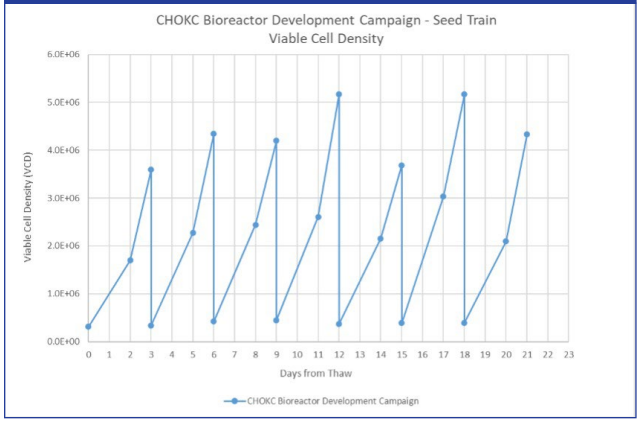
Parameters	Setting
Temperature 1 (°C)	37.0
Temperature 2 (°C)	Bioreactors BRX1 & BRX2 at 37, Bioreactors BRX3 & BRX4 from 37 to 32 on Day 6
Impeller Design	Three-blade segment, 45-degree, 70 mm
Number of Impellers	2 (set at 1 and 3 L)
Agitation (rpm)	150 (Tip Speed = 0.55 m/s)
DO (%)	30
pH	7.0 ± 0.2
pH high side control	CO_2 sparge
pH low side control	7.5% w/v $NaHCO_3$
CO_2 Sparge (mL/min)	20 mL/min max, variable, as needed for pH control
Air Sparge (mL/min)	Ring sparger, 14 holes, 0.5 mm, as needed for stripping CO_2 (pCO_2 >90mmHg turn on air sparge at 100 mL/min)
O_2 Sparge (mL/min)	100 mL/min, increase as needed for DO control
Production Medium	BalanCD CHO Growth A / 8mM L-Glutamine / 1% v/v Anti-Clumping Agent
Initial Working Volume (mL)	4000
Feed Supplementation	Cell Boost™ 7a at 1.0% Vi and Cell Boost™ 7b at 0.10% Vi days 4 through harvest
Glucose Supplementation	When < 4 g/L, feed to 6 g/L
Antifoam (FoamAway™ Irradiated AOF)[1]	As needed

delivered Cell Boost™ 7a at a rate of 1.0% the initial bioreactor volume (Vi) and Cell Boost™ 7b at a rate of 0.10% Vi on days 4 through harvest. The fed-batch bioreactor process was performed until Day 13. The clarified cell-free medium was saved for downstream process development work. Samples were collected during the fed-batch process for glucose, lactate, and titer assessment. Samples were analyzed with the Cedex Bio Analyzer (Roche CustomBiotech) for glucose, lactate, and IgG concentration.

Results

The viable cell density (VCD) during the various passages into the bioreactors are shown in FIGURE 2. On the first passage, the cells grew to a viable cell density of 3.5 x 10^6 cells/mL after three days of inoculation and they were split to a seeding density of 0.4 x 10^6 cells/mL for the second passage. The cells were passaged every three days when they grew to over 3 x 10^6 cells/mL and on the sixth passage, they were seeded into a 25 L wave bioreactor containing 5.5 L of medium. The cells in the wave bioreactor grew to over 5 x 10^6 cells/mL and these cells were used to seed the 4 x 5L bioreactors. Bioreactors BRX1 & BRX2 were run at 37˚C throughout while Bioreactors BRX3 and BRX4 were run at 37˚C until day 6 when the temperature was shifted to 32˚C.

FIGURE 2: Viable cell density (VCD) measured by the Vi-CELL XR Bioanalyzer.



"...performing the bioreactor run at 37°C throughout leads to a higher titer and a higher cumulative specific productivity."

All bioreactors were delivered Cell Boost™ 7a at a rate of 1.0% the initial bioreactor volume (Vi) and Cell Boost™ 7b at a rate of 0.10% Vi everyday starting from days 4 through harvest. When glucose levels fell below 4 g/L, glucose feed was supplemented to 6 g/L. The glucose levels were used to determine the point at which glucose needed to be added to the bioreactors. The glucose levels and the additions made during the campaign are shown in FIGURE 3. From FIGURE 3, glucose additions were made four times during the entire process. In the case of the CHOKC cells during this bioreactor campaign, lactate concentrations increased to maximal value of 3.37 g/L during the exponential growth phase

FIGURE 3: Glucose concentration measured using the Cedex Bio Analyzer.

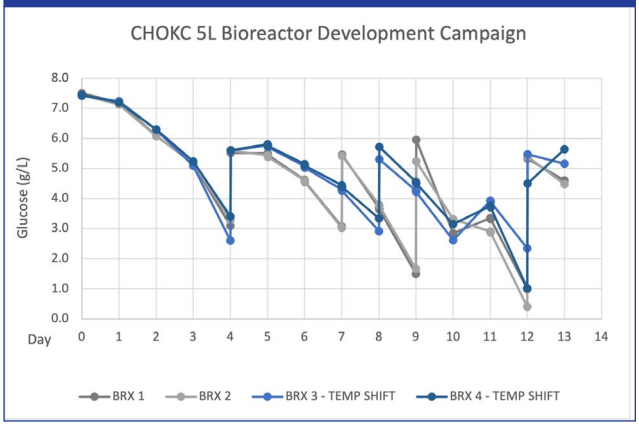
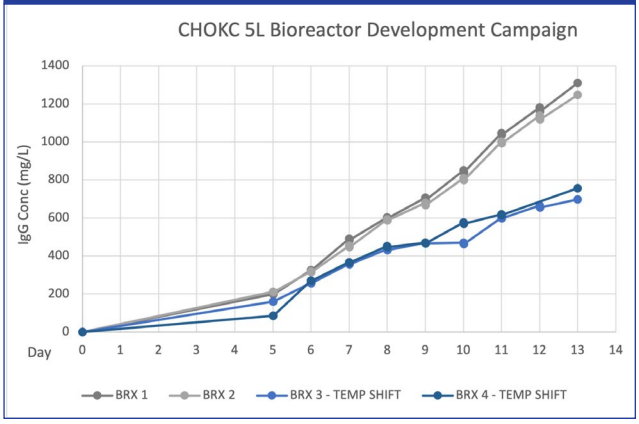


FIGURE 4: IgG titer measured by the Cedex Bio Analyzer.



and then decreased during the stationary phase with a final concentration between 1.16 and 2.95 g/L at the time of harvest.

The concentration of the trastuzumab was measured using the Cedex Bio Analyzer (IgG concentration). Bioreactors BRX1 and BRX2 with no temperature shift achieved the highest product titer of 1250 and 1310 mg/L and had an average cumulative specific productivity of 6.34 pg/cell/day at the time of harvest. Bioreactors BRX3 and BRX4 with a temperature shift to 32˚C achieved the lowest product titer of 697 mg/L and 755 mg/L and had an average cumulative specific productivity of 3.87 pg/cell/day at the time of harvest. The results, FIGURE 4, clearly indicate that performing the bioreactor run at 37˚C throughout leads to a higher titer (76% improvement) and a higher cumulative specific productivity (64% improvement) compared to performing a temperature shift. There was no difference in the viable cell density between the bioreactors with and without a temperature shift. The harvested samples will be taken through purification and analyzed for product attributes to ensure they meet product specifications.

Summary

A mammalian platform process for the expression of therapeutic proteins is being developed using the CHOKC cell line in a fed-batch cell culture process. The preliminary results from this work are presented here. The Vi-Cell XR Bioanalyzer from Beckman Coulter was used to measure the VCD for each split and inoculation of the bioreactors. The Cedex Bio Analyzer from Roche CustomBiotech was used to measure glucose and when the glucose levels fell below 4 g/L, glucose supplementation

was provided to bring glucose levels up to 6 g/L. The Cedex Bio Analyzer also provided information on titer of the product measured by IgG concentration. The results showed that the platform process being developed for the CHOKC platform has a higher titer when the temperature is maintained at 37˚C throughout compared with a temperature shift to 32˚C. Currently, additional data is being collected on purified protein from the bioreactors to ensure the IgG protein meets product quality attributes. In addition to trastuzumab, other therapeutic proteins need to be evaluated.

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Trademarks and Disclaimers

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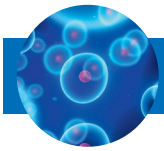
Powerful, flexible and comprehensive cell culture profiling

Cedex Bio and Cedex Bio HT Analyzers



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Use Case: A Smart Path for Novel Biologics

Pawel Linke and Markus Neubauer

How Roche Pharma increased the number of projects in process development without a significant impact on resources

INCREASE THE NUMBER OF PROJECTS IN PROCESS DEVELOPMENT WITHOUT SIGNIFICANTLY INCREASING HEADCOUNT. PAWEL LINKE, A LAB AUTOMATION SPECIALIST AT ROCHE PHARMA, TELLS THE STORY OF HOW ROCHE PHARMA RESEARCH AND EARLY DEVELOPMENT (PRED) APPROACHED THIS CHALLENGE WITH A FOCUS ON OBTAINING HIGH QUALITY DATA THROUGH AUTOMATION AND BY INTEGRATING INDEPENDENT LAB SYSTEMS AND DEVICES THROUGH A STREAMLINED WORKFLOW.

IMPLEMENTATION OF HIGH-THROUGHPUT UPSTREAM PROCESS DEVELOPMENT SYSTEMS REQUIRES AN ORCHESTRATED APPROACH OF BOTH HARDWARE AND SOFTWARE SOLUTIONS SO THAT DATA CAN BE USED IN-PROCESS, ENABLING EFFICIENT DEVELOPMENT AND DECISION MAKING. THIS USE CASE DISCUSSES THE INTEGRATION OF PARALLEL MICROBIOREACTORS, ROBOTIC LIQUID HANDLING (WITH TECAN FLUENT), AND THE CEDEX BIO HT ANALYZER FOR BIOPROCESS CONTROL AS WELL AS THE MESH-LIKE MIDDLEWARE THAT SEAMLESSLY MANAGES THE DATA FLOWING AMONG EACH OF THE LAB INSTRUMENTS. ALL OF THESE

USE CASE: A SMART PATH FOR NOVEL BIOLOGICS

SYSTEMS WORK IN HARMONY TO INCREASE THE NUMBER OF THERAPEUTIC PROTEINS DEVELOPED AT pRED. WITH AN EYE ON THE FUTURE, ROCHE PHARMA SEEKS TO TAKE THESE LEARNINGS AND APPLY THEM TO BIOLOGICS MODALITIES, SUCH AS GENE THERAPY, MAKING THIS WORKFLOW SOLUTION A SMART PATH FOR NOVEL BIOLOGICS.

Introduction

Cell culture is at the heart of the production process for many biopharmaceuticals, but finding the optimal conditions to maximize yield can be a complex and time-consuming process. Traditional process development relies on costly and labor-intensive set-ups, significantly limiting throughput and the range of experimental conditions that can be assessed. Scientists in Roche Pharma Research and Early Development (pRED) have adopted an alternative approach, combining single-use microbioreactors with advanced automation and analytical platforms to streamline the workflow.

SPONSORED CONTENT

In-lab Demo: Operating the Cedex Bio HT Analyzer

Discussion

Optimizing fermentation takes time; and people

The production of large biological molecules for therapeutic use is complex, requiring expression of the target peptide, protein or antibody in suitable host systems, followed by extraction and purification to yield the final drug. Efficient expression requires not just the right host organism and clone, but also the right physical conditions and chemical environment to promote production.

Exploring these numerous parameters to optimize biotherapeutic production is a very time-consuming complex process, requiring cell expansions in the seed train to subsequently run numerous fermentations in parallel to identify the best candidate and its conditions for expression. With more data points being obtained for each variable, it's more likely to establish a highly productive final process. Fermentation development has historically relied on performing parallel experiments using a number of shake flasks or 2 L bioreactors, with continuous monitoring via an array of samples from each of these bioreactors to enable analytics. Unfortunately, this approach is both time consuming and labor intensive, with one researcher able to handle only a few 2 L bioreactors simultaneously.

USE CASE: A SMART PATH FOR NOVEL BIOLOGICS

SPONSORED CONTENT

Cedex Bio HT Analyzer 96-well plate upgrade

A microbioreactor farm—Miniaturized, parallelized condition testing

When the Bioprocess Research Department at the Roche site in Penzberg, Germany, was challenged with significantly increasing numbers of proteins to be produced, the team realized it needed to look for an alternative solution. Pawel Linke, specialist for lab automation, explained: "We were asked by the management to look for a way to do even more process development for the more complex bio-therapeutic candidates, as well as clearly increasing the number of projects we performed per year. This clearly wasn't going to be possible with our existing workflow—a very hands-on process—without significantly increasing headcount, which wasn't an option.

"We looked at the possible solutions on the market and chose a system of high-throughput single-use microbioreactors. We now have two different types of microbioreactors: four that can run up to 48 parallel fermentations of up to 15 mL, and five larger vessels that allow us to run 12 x 250 mL fermentations simultaneously. This shift represented a huge increase in capacity, allowing us to run four or five times as many fermentations with the same number of staff. However, it also created new challenges, as we significantly decreased the culture volume while at the same time increasing the number of samples to be analyzed. We therefore needed a way of efficiently processing up to 250 samples every day, before analyzing them on a Cedex Bio HT Analyzer from our sister division Roche Diagnostics CustomBiotech.

"We have a huge number of laboratory automation systems here in Penzberg and, based on this experience, we decided that a Tecan liquid handling platform would be the best fit for our needs. A key requirement for our workflow was the ability to integrate a large centrifuge to spin down the samples before analytics, and the Fluent® Automation Workstation's ability to mount a robotic centrifuge below the worktable was a real benefit. We simply place the samples from the bioreactors on the workdeck, and the system is able to automatically load them into the centrifuge using its Robotic Gripper Arm™ (RGA). Once spun down, the Fluent platform splits the supernatant into several aliquots in different labware formats, depending on the downstream application.

"We now have two different sized Fluent systems with slightly different configurations, giving us more than enough capacity to process samples for every microbioreactor every day, although we have this many experiments running in parallel at present. The smaller system is equipped with an RGA for labware movements and an 8-channel Liquid Handling Arm™ (LiHa) for pipetting, while the larger system has a third arm

equipped with a Multiple Channel Arm™, allowing entire 96-well microplates to be duplicated or transferred simultaneously. Each platform also has on deck integrated Tecan microplate readers for some onboard analytics. Although both have cooling modules, we have now developed for mass spectrometry analytics a workflow based on sealed, pre-pierced plates that can be accessed by the LiHa. This avoids evaporation without the need for cooling, further simplifying the workflow."

Joint efforts to let components interact the most efficient way

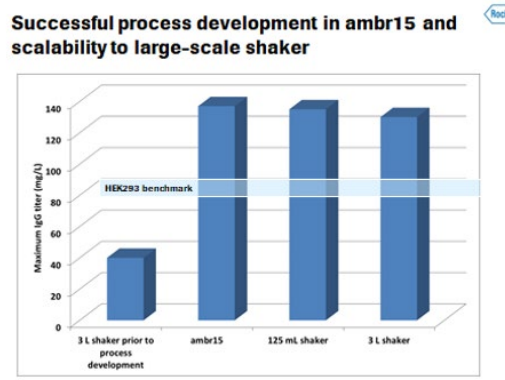
"When we first implemented this set-up, the Cedex Bio HT Analyzer required 15 samples to be loaded into a rack for processing, but we have worked with our Roche Diagnostics CustomBiotech colleagues to develop a 96-well microplate-based sampling system, allowing us to more efficiently and accurately transfer the high number of samples between the devices," said Peter Hloch, Director International Product Management, Roche CustomBiotech. He highlighted: "Automation is key in the future of bioprocessing, and joint efforts are needed to let components interact in the most efficient way. It was our pleasure to support the colleagues in pRED. Finally we developed an optimal solution in record time that is now ready for all our Cedex Bio HT users.

"Furthermore, we implemented Sm@rtLine Data Cockpit (SDC), a middleware from AGU GmbH to streamline the flow of online-, atline- and metadata between all devices. This solution is able to take the sample IDs from each microbioreactor and provide that information to Tecan's FluentControl™ software. The Fluent platform uses an integrated barcode scanner to confirm the identity of each sample, associates that ID to all aliquots it generates for the analysis, and exports the information back to SDC. The middleware then provides the relevant information to the Cedex Bio HT Analyzer. This means that, although each platform is effectively a standalone instrument, the software environment is as automated as the physical process—it's a very smooth workflow finally allowing a feedback loop to the bioreactor.

Cedex Bio HT Analyzer

USE CASE: A SMART PATH FOR NOVEL BIOLOGICS

"We developed this approach because we have many different researchers in the lab, working on a number of different projects. The touchscreen user interface on the Fluent workstations makes it very easy for people to walk up and use the systems with only minimal training—we have around 30 users—with a couple of key users' to handle protocol development and maintenance tasks. All together it's a great fit to our needs."



Transient expression levels of an IgG antibody prior to process development using automated microbioreactor systems at 15 mL scale (ambr15) and after successful process development resulting in higher yields. The results of ambr15 process development could be transferred back and upscaled to shaker flask cultures in 125 mL and 3 L scale.

Conclusion

Roche pRED established a highly automated high-throughput workflow in cell culture development for new antibody drugs. The pillars of this workflow are automated microbioreactor systems (Ambr®, Sartorius) in the 15 mL and 250 mL scale, pipetting robots (Tecan Fluents), and the analytical devices, such as the Cedex Bio HT Analyzer (Roche CustomBiotech). The Ambr® systems serve as working horses for upstream process (USP) development and cell line selection supporting fast and lean development. Tecan pipetting robots are used for liquid handling in sample processing, and the Cedex Bio HT Analyzer for cell culture monitoring (with the Cedex Bio HT Analyzer being linked to Ambr® via Tecan Fluents). As shown below, we proved this workflow to be highly efficient for antibody development, especially by taking advantage of the 96-well sample loading format on the Cedex Bio HT Analyzer, and data management with Sm@rtLine Data Cockpit. This workflow was a key enabler of USP platform evolution, development of complex antibody formats at high quality, and acceleration of molecules to market, into clinics, and ultimately to patients.

Transient expression levels of an IgG antibody prior to process development using automated microbioreactor systems at 15 mL scale (Ambr® 15) and after successful process development resulting in higher yields. The results of Ambr® 15 process development could be transferred back and upscaled to shaker flask cultures in 125 mL and 3 L scale.

Future Outlook

Leveraging Bioprocess Development workflows of therapeutic antibodies for new biologics modalities

In recent years, new biologics modalities are emerging including gene therapy modalities. Roche pRED Bioprocess Leader, Markus Neubauer, explains, "We are working on adeno-associated virus (AAV) vectors as gene therapy modality. State-of-the-art manufacturing technology is still triple transfection of human cell lines whereas stable cell lines are envisioned as future manufacturing technology for AAV vectors

by us and many others in the field. We successfully applied the automated workflow described above for transient transfection of human cell lines with antibody-coding plasmids.[1] Currently, we are re-purposing this workflow for triple transfection of human cell lines with AAV plasmids. In the future, we see a perfect fit of the Ambr®-Tecan-Cedex workflow for application with stable cell lines to develop AAVs manufacturing process in an industrial workflow and scale. This technology will be a key enabler for high volume indications in the field of gene therapy."

Pawel Linke

Scientist Lead Identification & Lab Automation
Roche Pharma

Markus Neubauer, PhD

Head of Bioprocess Research
Roche Pharma

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1. Neubauer, Markus. Development of efficient transient transfection processes for different cell lines using an automated and miniaturized bioreactor system. PEACe conference 2015, Talk 4.2, pg. 47. https://www.conferium.com/Clients/PEACe/PROGRAM_12PEACe.pdf. Accessed: 25 May 2021.

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Doing More with Less: Maximizing Phase Appropriate DOE Strategies - Keynote Summary

THIS ARTICLE SUMMARIZES A KEYNOTE PRESENTATION FOR THE LABROOTS 2021 BIOPROCESSING VIRTUAL EVENT BY PATRICIA D. MCNEILL, ASSOCIATE DIRECTOR, CELL CULTURE DEVELOPMENT, LUNDBECK SEATTLE BIOPHARMACEUTICALS, INC.

Brandy Sargent

Combining definitive screening designs with advanced machine learning benefits for accelerated drug development.

ONE CHALLENGE PHARMACEUTICAL COMPANIES FACE IS MOVING PROMISING BIOTHERAPEUTIC PRODUCTS INTO THE CLINIC AS FAST AS SAFELY POSSIBLE. DEVOTING THE CORRECT AMOUNT OF TIME AND RESOURCES TO EARLY-STAGE PRODUCTS CAN STREAMLINE THE LATE STAGE AND COMMERCIAL DEVELOPMENT ACTIVITIES. A CELL LINE CHANGE BETWEEN PHASE I/II AND PHASE III CAN RESULT IN SPENDING SIGNIFICANT QUALITY AND REGULATORY RESOURCES TO DEMONSTRATE COMPARABILITY BETWEEN THE 1ST AND 2ND GENERATION PRODUCTS. EARLY DOE SCREENING STUDIES CAN SELECT CELL LINES THAT HAVE THE MOST ROBUST MANUFACTURABILITY PROFILE, PERHAPS NEGATING THE NEED FOR A CELL LINE SWITCH. AS THE PROJECT PROGRESSES, PROCESS CHARACTERIZATION STUDIES CAN BE VERY LABOR INTENSIVE AND ARE USUALLY DELAYED UNTIL A PRODUCT HAS PROVEN ITSELF WORTHY OF INVESTMENT THROUGH SUCCESS IN EARLY CLINICAL TRIALS.

DEFINITIVE SCREENING DESIGNS[1] HAVE THE POTENTIAL TO BE A ONE-STOP SHOP FOR SCREENING IMPORTANT PARAMETERS AND OPTIMIZING

SETTINGS WITH LOW RESOURCE COSTS. ADVANCED MACHINE LEARNING METHODS HAVE REVOLUTIONIZED THE ANALYSIS OF LARGE DATA SETS WHERE THE DATA CAN BE DIVIDED INTO PORTIONS FOR TRAINING, TESTING AND VALIDATION. DURING BIOPROCESSING DEVELOPMENT, THE DATASETS ARE USUALLY SMALL AND UNTIL NOW HAVE NOT BEEN SUITABLE FOR PORTIONING THE DATA INTO DIFFERENT TRAINING AND VALIDATION SETS. THE FRACTIONALLY WEIGHTED BOOTSTRAPPING METHOD[2] USES THE SAME DATA SET FOR TRAINING AND VALIDATION. THIS AUTO-VALIDATION TECHNIQUE HAS BEEN EMPLOYED TO SUCCESSFULLY DE-RISK MODEL TESTING EXPERIMENTS THAT COMPLETE THE DOE LIFECYCLE.

Introduction

As a result of several factors, including speed to market initiatives, pressure has been increasing to shorten the drug development timeline in an effort to maximize efficiency, reduce cost, and achieve first to clinic advantage. As a drug product candidate moves through the development timeline from pre-clinical research to late stage and finally commercial development, there is an increased need for resources and personnel, which requires additional cost and time. Thus, balancing the effort and resources required for early- and late-stage development with the need to maximize speed and efficiency is critical.

One way to maximize efficiency and still acquire the data needed to make important development decisions is through the use of Design of Experiment (DOE) Strategies. Ideally, DOE provides

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Keynote Presentation: Doing more with less - Maximizing Phase Appropriate DOE Strategies

experimental strategies that will extract the maximum amount of information from the least number of runs. In early development, screening designs are excellent for identifying which parameters are most significant in affecting product outcome (yield and product quality). In late stage and even into post-launch activities, characterization studies can be used to gain greater process understanding and even to predict the outcome of specific parameter settings in the design space. These insights can then be applied for a greater understanding of parameter interactions that can guide process optimization, identification of critical process parameters (CPP)s, and critical quality attributes (CQA)s for the manufacturing process. DOE also permits faster development timelines compared to One Factor at a Time (OFAT) experiments, which are time and labor intensive.

Phase-Appropriate DOE Strategies

Patricia D. McNeill, Associate Director, Cell Culture Development at Lundbeck Seattle BioPharmaceuticals, recently hosted a webinar on her use of DOE strategies to maximize speed and efficiency of drug development. Ms. McNeill presented case studies from her work at Lundbeck, a global pharmaceutical company

specialized in discovering and developing innovative treatments for brain diseases. These studies were designed to maximize screening for early-phase development and characterization during late phase development. Her use of DOE and auto-validation for small data sets coupled with the rapid, reliable analytics and powerful modeling software provided a road map for early- and late-stage development that saved time and resources.

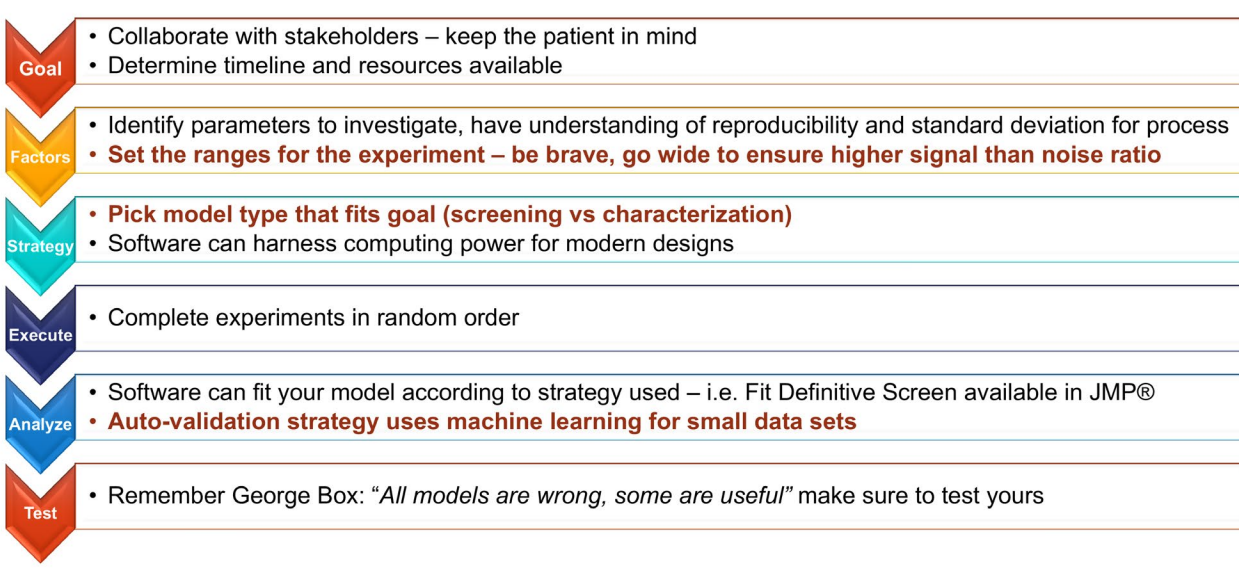
Drug Development and Key CMC Milestones

Ms. McNeill began by explaining that for pre-clinical, the focus is on achieving first in human process. Thus, in this stage, it is important to choose a robust cell line to get the product into the clinic, but it doesn't need to represent the final process. Choosing the right cell line is important because changing the cell line represents a major change that requires vetting and the additional resources of quality and regulatory groups to ensure patient safety. Making that kind of change requires time and resources, thus it is best to avoid this situation by selecting the right cell line upfront.

During Phase I clinical trials, the company should be initiating commercial process development and this means optimizing for yield and product quality. At this stage, companies will be developing the process for Phase III. The process for Phase III should be the same as used for commercial launch. After the process is developed, process characterization should be conducted to gain a better understanding of the operating space and to fully validate the commercial process. The final goal is a validated commercial process that will support robust product manufacturing.

Implementing a DOE Approach

FIGURE 1: Implementing a Design of Experiment approach.



Ms. McNeill shared a nice graphic of the steps involved in implementing a DOE approach (FIGURE 1).

During the discussion, she stressed the importance of first talking to cross-functional stakeholders to determine the timeline and resources available. For instance, she shared that during one project, while talking to stakeholders, the team realized that the antibody in development was sticking to the tubing and thus not manufacturable. As a result, they were able to switch to their second candidate choice, which saved the project and allowed the product to be successfully launched.

Next, it is important to identify the factors for investigation and to have a good understanding of the reproducibility and standard deviation of the process. She recommended setting wide ranges for the experiments to ensure higher signal than noise ratio to create more useful models.

Then, you need to choose the strategy that best fits the goal (i.e., screening vs. characterization) and execute the experiments in random order. Using modeling software, the results can be analyzed and an auto-validation strategy implemented to apply machine learning for small data sets. Last, be sure to test the model as each run can be expensive and validating the model will offer the highest chance of success.

Pre-DOE homework

To ensure success, Ms. McNeill strongly recommended doing your pre-DOE homework. The pre-DOE homework is important as it provides an understanding of process variability prior to DOE. The first thing that they evaluated was the variation in process—between scales (250 mL Ambr®, 3 liter and 14 liter single-use bioreactors), between users, and between run sets. They found good consistency across all areas.

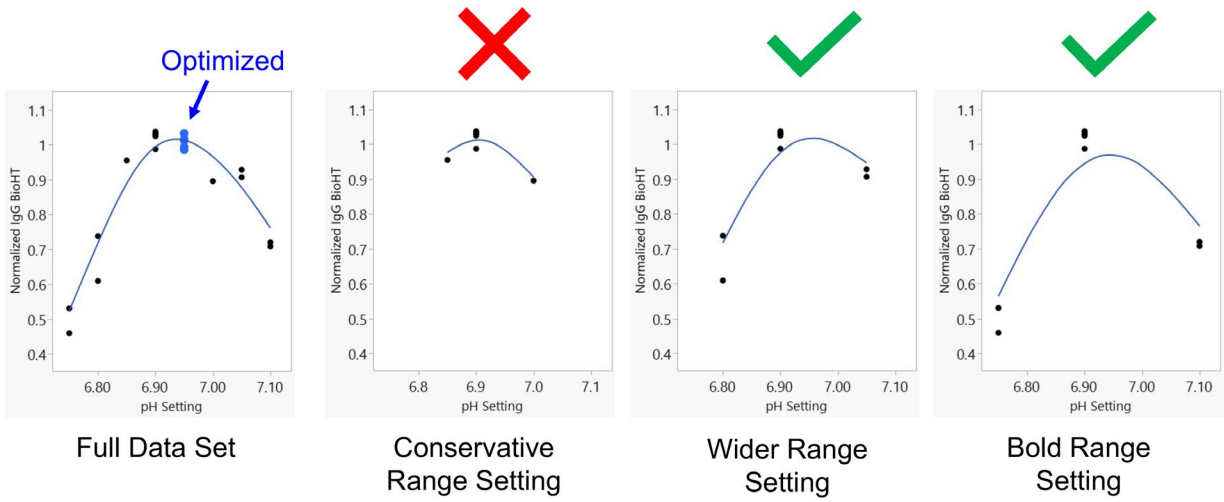
Next, they looked at variation in analytics—between instruments, reference lots, and analyzers. Looking at the variation in analytics is important for setting experimental factor ranges. The ranges must be wide enough to create a useful model that can enable informed decisions moving forward, increased signal-to-noise ratios create more useful models.

Examples of how to set ranges

Ms. McNeill explained that there are options when it comes to setting ranges during DOE. She presented examples of a full data set, a conservative data range, a wider range setting and a bold range setting (FIGURE 2). She explained that the conservative range setting did not identify the optimized setting found with the full data set. Using the wider range setting did identify the mathematically optimized condition and so did the bold

"The final goal is a validated commercial process that will support robust product manufacturing."

FIGURE 2: Setting factor ranges.

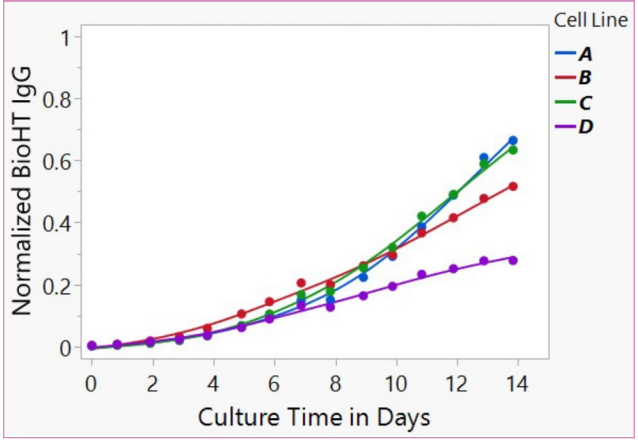


range. Thus you do not need to test every parameter, but you do need to set a range that is wide enough.

Case Study: Early-Stage Development

Screening studies can be used to select the best performing cell line. In this case study, they used the Cedex Bio HT Analyzer to measure titer at several time points in the run (FIGURE 3). In addition to yield, they evaluated product quality, manufacturability, and stability - both genetic stability over several passages and product stability over time and temperature.

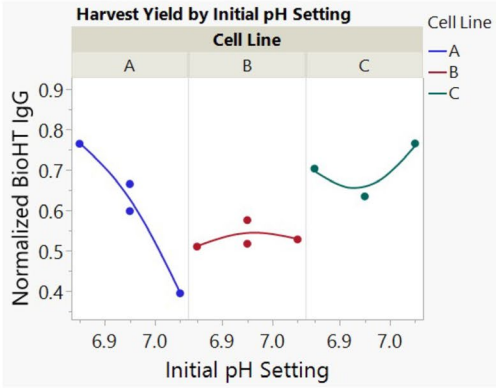
FIGURE 3: Normalized IgG Titer (Cedex Bio HT)



Explore manufacturability

In FIGURE 4, Ms. McNeill explained the importance of looking at manufacturability of the cell line. Yield, while an important factor, is not the only consideration. It is important to investigate the top process factors (e.g. yield and pH) for the top performing cell lines. She pointed out that balancing yield with cell line robustness across a moderate process range is important in selecting the correct cell line early. For example, in FIGURE 4, cell line A has the highest titer, but the lowest manufacturability, because minor changes in pH caused a significant drop in

FIGURE 4: Explore manufacturability.



- ***Investigate top 2 process factors with top 3 cell lines***
- ***Balance desirability of response with robustness across moderate process range***
- ***Finding correct cell line early saves time and resources later***

Cell Line	Result
A	Highest Titer, Lowest Manufacturability
B	Lowest Titer, Highest Manufacturability
C	High Titer, Ok Manufacturability

titer. Cell line B had the lowest titer, but it had the highest manufacturability. Cell line C had high titer and OK manufacturability. It is important to weigh titer vs. manufacturability to find a cell line that will work for the process. However since it isn't the final process, titer could be increased using other process factors. On the other hand, manufacturability issues can force a company to have to change cell lines altogether.

Case Study: Late-Stage Development

Next, Ms. McNeill shared a case study from late stage upstream development. She explained that while the product is in clinical trials, it is important to conduct late stage process development to develop the Phase III process.

Key activities include:

- Perform a risk assessment to identify key factors that need screening. Be sure to avoid prior assumptions, treat this like a new cell line and new antibody.
- Choose an experimental design approach that best fits the desired data.

They chose a Definitive Screening Design (DSD) approach for optimization because it is efficient for screening four or more parameters. It is capable of investigating low, middle, and high settings for continuous factors, which allows fitting of a curve. It can also include two-level categorical factors, (i.e., two different nutrient feeds), and can account for block effects (i.e. different run sets). Brad Jones and Christopher Nachtsheim developed this approach and a talk is available, which describes this method in more detail.

After the design approach was selected, the next step was to meet with teams to identify the top ten terms for further investigation.

In FIGURE 5, the selected terms are listed across the top. The Lundbeck team wanted to compare their platform process against DSD modeling of conditions that weren't

FIGURE 5: Comparing Lundbeck's platform process with DSD modeling of conditions.

Block	N-1 Seed Density	Seeding Density	Starting Temp C	pH	Temp Shift	Glucose	Feed A	Feed B	Feed Start	Temp Shift Day
1	0.9	1	36	6.95	34.5	6.5	5	0.5	72	7
1	0.9	0.5	34	6.75	38	10	3	0.7	48	10
1	1.3	1.5	38	6.95	38	10	7	0.7	96	10
1	0.5	0.5	38	7.15	38	6.5	3	0.3	96	10
1	1.3	1.5	34	6.75	31	6.5	7	0.7	48	4
1	0.5	0.5	34	6.95	31	3	3	0.3	48	4
1	0.9	1.5	38	7.15	31	3	7	0.3	96	4
2	0.5	1	38	6.75	38	10	7	0.3	48	4
2	0.5	1.5	38	7.15	31	10	3	0.7	48	7
2	1.3	1	34	7.15	31	3	3	0.7	96	10
2	0.9	1	36	6.95	34.5	6.5	5	0.5	72	7
2	0.5	1.5	34	6.75	31	10	5	0.3	96	10
2	1.3	0.5	34	6.75	38	3	7	0.3	96	7
2	1.3	0.5	38	7.15	38	3	5	0.7	48	4
3	0.5	1.5	36	6.75	38	3	3	0.7	96	4
3	0.5	1.5	34	7.15	38	3	7	0.5	48	10
3	1.3	0.5	38	6.75	31	10	3	0.5	96	4
3	0.9	1	36	6.95	34.5	6.5	5	0.5	72	7
3	1.3	0.5	36	7.15	31	10	7	0.3	48	10
4	0.5	0.5	38	6.75	31	3	7	0.7	72	10
4	0.9	1	36	6.95	34.5	6.5	5	0.5	72	7
4	1.3	1.5	38	6.75	34.5	3	3	0.3	48	10
4	0.5	0.5	34	7.15	34.5	10	7	0.7	96	4
4	1.3	1.5	34	7.15	38	10	3	0.3	72	4

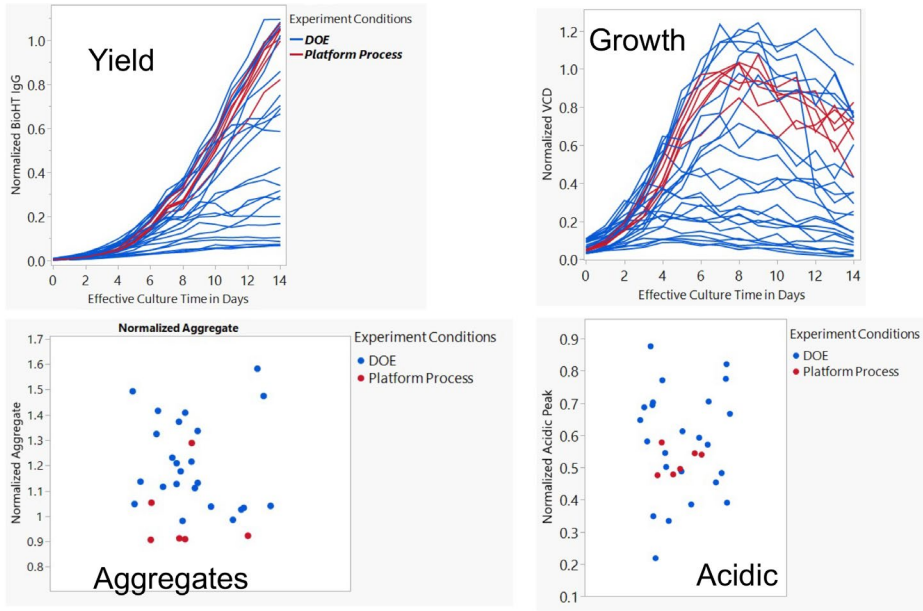
tested yet to see if they could increase titer. At the end of 24 bioreactor runs, some of the ten terms limited growth and some increased growth, thus they knew that with further study they would be able to find factors that improve yield and growth.

DSD results compared to platform process

3 Key takeaways based on results in FIGURE 6:

1. Wide variation for yield and growth in DOE compared to variation in platform process means bold range setting achieved desired result.
2. DOE results for aggregates were similar to results from the platform process, which means that without any further experimentation, they know the platform process is fairly robust for this quality attribute across all ten factors tested.
3. DOE results for charged species have wide variation compared to platform process, thus at least one parameter has a strong impact within the range tested.

FIGURE 6: DSD results compared to platform process.



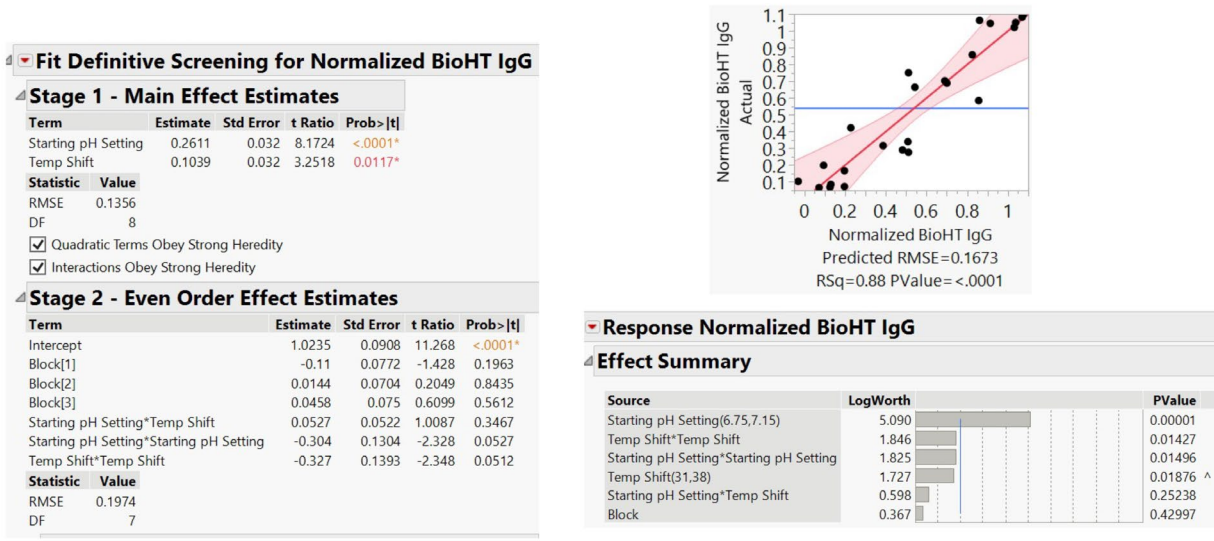
Analyzing results using modeling software
Ms. McNeill provided an overview of the process for analyzing study results using modeling.

- **Stage 1:** Look at the top ten parameters and how much changing each of these affects titer. The top two parameters identified as impacting the culture were starting pH and temperature shift.
- **Stage 2:** Look at the remaining information not explained by changing the top ten factors. Review the parameter interactions and check if there is curvature within parameters. Results showed that again starting pH and temperature shift had the most impact on culture performance.
- **Stage 3:** Combine stage 1 and stage 2 to create a mathematical model to describe the response.

Modeling options
Analyzing results using the JMP® modeling software provides several model options:

- **Fit Definitive Screen Option 1:** In this model the quadratic terms obey strong heredity and interactions obey strong heredity. This means that if the main parameter did not have a strong response on titer, then it wouldn't be included in a curvature term or interaction with any other factor. This is a simple model with less chance of over fitting. A parietal plot was used to rank the effect summary and

FIGURE 7: Analyzing Results—Fit Definitive Screen Option 1.



in this option, the top two parameters identified as impacting the culture were starting pH and temperature shift. An example of the layout for option 1 can be seen in FIGURE 7.

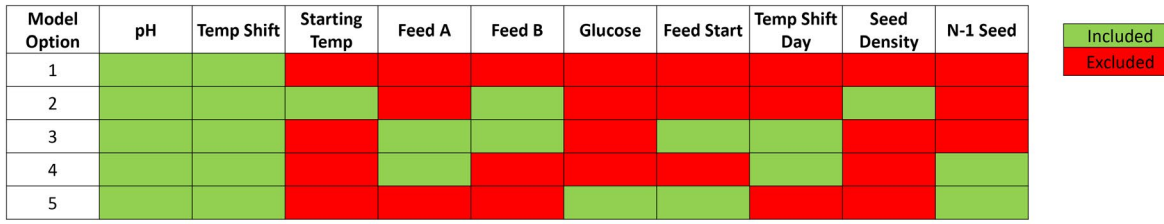
- **Fit Definitive Screen Option 2:** In this model the quadratic terms don't have to obey strong heredity; there might be curvature for a parameter that is more important than the parameter itself. The parietal plot results for this option, identified the starting pH at the top effect with temperature shift 2nd, but production seed density and Feed B percentage both show curvature.

- **Fit Definitive Screen Option 3:** Here, quadratic terms obey strong heredity, but interactions do not. In this model an interaction between starting pH and Feed B percentage was identified. Also, there was an interaction with nutrient feed start time in hours and Feed A. Starting pH and temperature shift were still identified as top parameters affecting titer.

- **Fit Definitive Screen Option 4:** In this option, all possible combinations can be included with no restrictions on quadratic or interaction terms. Results showed that starting pH was still the most important parameter.

- **Stepwise Forward Minimum AICc Option 5:** In this model, all ten factors are added to model terms as response service, which means that for all ten factors, all interactions and all quadratic terms will be looking for IgG titer. This is achieved by adding Y response as IgG titer. Results showed that starting pH

FIGURE 8: Comparing model options.



and temperature shift were still at the top, but glucose target had some curvature.

So what to do with all of this information? Compare the models and select the best to use. Ms. McNeill described this important step to ensure that what you are testing is going to accurately predict the method you should move forward with. In looking at the models (FIGURE 8), all found that pH and temperature shift were important main effects impacting titer. The remaining eight parameters tested were included in some, but not all models.

Auto-validation for small data sets

Machine learning methods have revolutionized analysis of big data sets, but can also effectively be used for small data sets. For instance, Ms. McNeill stated that you can use a fraction of the data for training or building the model, a fraction for validation and a fraction for testing and all can be done *in silico*. She recommended a talk on Model Validation Strategies for Designed Experiments from the JMP Discovery Summit by Chris Gotwalt, JMP® Division SAS Institute and Phil Ramsey, University of New Hampshire to provide more details on validation strategies.

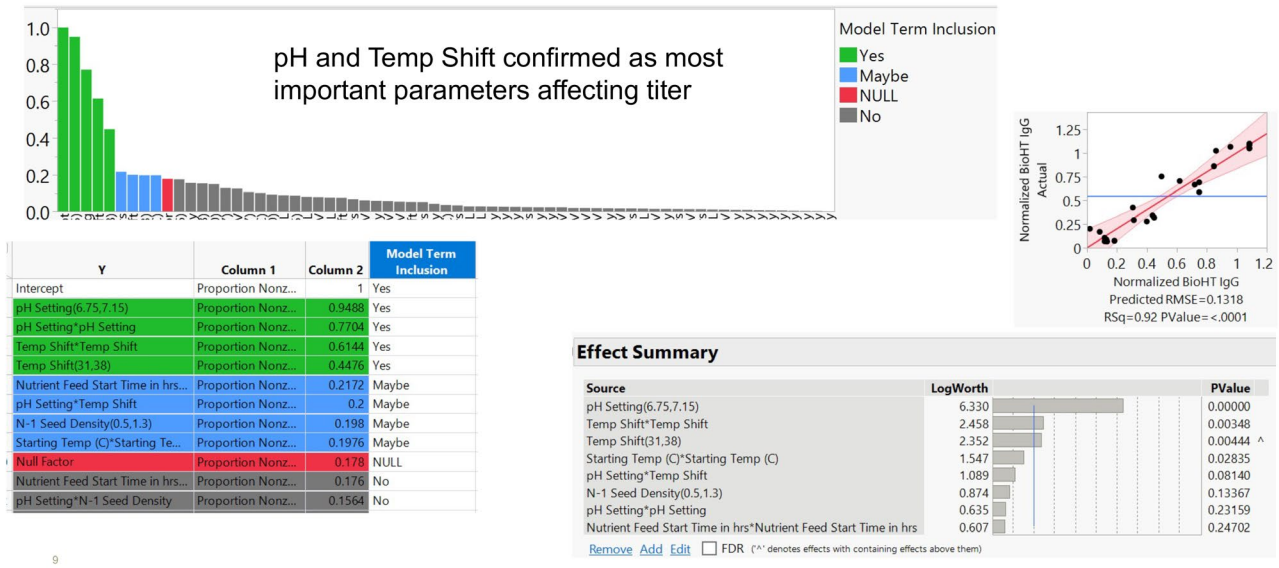
The strategy that she chose uses the Fractionally Weighted Bootstrap method in combination with the "Null Factor" to identify model terms. It uses the same data for training as for validation and requires JMP® Pro software.

Set up data for analysis

Ms. McNeill explained that the JMP®Pro software will incorporate the necessary columns to conduct the fractionally weighted bootstrap method. This involves adding three new columns, by creating a copy of the original DOE data set and adding a validation column. Then, to the original data set, you add a "0", which will be the training set. To the copied data set you add "1", this will be the validation set. Next, you create a new formula column for the Fractionally Weighted Bootstrap, and a new column for "Null Factor". The Null Factor is a randomly distributed result independent of monitored response such as titer. It functions as a random number that will change each time you run a simulation.

The Lundbeck team performed 2,500 simulations (FIGURE 9). Using the data generated, they were able to identify the relevant model terms by comparing them to

FIGURE 9: Identify model terms by comparing to null factor.



Y	Column 1	Column 2	Model Term Inclusion
Intercept	Proportion Nonz...	1	Yes
pH Setting(6.75,7.15)	Proportion Nonz...	0.9488	Yes
pH Setting*pH Setting	Proportion Nonz...	0.7704	Yes
Temp Shift*Temp Shift	Proportion Nonz...	0.6144	Yes
Temp Shift(31,38)	Proportion Nonz...	0.4476	Yes
Nutrient Feed Start Time in hrs...	Proportion Nonz...	0.2172	Maybe
pH Setting*Temp Shift	Proportion Nonz...	0.2	Maybe
N-1 Seed Density(0.5,1.3)	Proportion Nonz...	0.198	Maybe
Starting Temp (C)*Starting Te...	Proportion Nonz...	0.1976	Maybe
Null Factor	Proportion Nonz...	0.178	NULL
Nutrient Feed Start Time in hrs...	Proportion Nonz...	0.176	No
pH Setting*N-1 Seed Density	Proportion Nonz...	0.1564	No

the Null Factor. First, the percentage of time the null strain was randomly included to be a factor is detected. Because we know that the Null Factor isn't important, it is random noise, every model term that is included less than the Null Factor is also not important. Looking at FIGURE 9, the red bar represents the Null Factor, so everything below that (gray shaded lines) are not important model terms. Green is highly important and blue is slightly more than the Null Factor.

Deciding which is the best model to use: Analyzing results and comparing models

Again, the Lundbeck team plotted all the models including auto-validation results (FIGURE 10). All models found pH and temperature shift to be important main effects, the remaining eight parameters tested were included in some models, but not all. They found three additional factors that might also be important (starting temperature, feed start, and N-1 seed) and tested them with wet lab experiments. As a result, they did find that starting temperature was also an important parameter.

Test the model's predicted optimal process

The final step was to test the model to determine if the auto-validation method was useful. They used the auto-validation method for each response of interest: growth, yield, and product quality. Each response received its own individual model with prediction models saved to the data table. They used Prediction Profiler in JMP® to find the optimized settings for the combined responses. The auto-validation model was tested in duplicate and results were as predicted, within the 95% individual confidence interval.

FIGURE 10: Comparing auto-validation to other models.

Summary

DOE and auto-validation for small data sets are key tools for early- and late-stage development that can save time and resources and ultimately speed time to market. Common pitfalls include not being bold enough when selecting the DOE factor ranges and using experimental designs that do not align with the goals of the experiments. Selecting the appropriate DOE approach that fits the goals for the current phase of development as well as utilizing a bold experimental design, enables a streamlined development process.

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2. Ramsey, Phil and Gotwalt, Chris 2017 Model Validation Strategies for Designed Experiments [Power point Slides] Fall Technical Conference https://falltechnicalconference.org/wp-content/uploads/2018_Presentations/3B-FTC-Ramsey-Final.pdf

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Brandy Sargent
Editor-in-Chief
Cell Culture Dish & Downstream Column

Phase Appropriate DOE: An Interview with Patricia D. McNeill

Avoid upstream and downstream silos to ensure a process is robust, reproducible and manufacturable.

One challenge pharmaceutical companies face is moving promising biotherapeutic products into the clinic as fast and as safely possible. Devoting the correct amount of time and resources to products in early stages can streamline late-stage and commercial development activities. Early Design of Experiments (DOE) screening studies can select cell lines that have the most robust manufacturability profile, perhaps negating the need for a cell line switch. As the project progresses, process characterization studies can be very labor intensive and are usually delayed until a product has proven itself worthy of investment through success in early clinical trials. *BioPharm International* recently spoke with Patricia D. McNeill, Associate Director of Cell Culture Development at Lundbeck Seattle BioPharmaceuticals, Inc., about the potential of Definitive Screening Designs (DSD) to screen parameters and optimize settings with low resource costs. She also discussed using advanced machine learning methods that have typically been reserved for large data sets as well as using the auto-validation

technique, which relies on the fractionally weighted bootstrap method, to apply machine learning to smaller DOE data sets in order to de-risk model testing experiments that complete the DOE life cycle.

BIOPHARM INTERNATIONAL: **What does maximizing phase-appropriate DOE strategies mean?**

MCNEILL: Performing DOE has traditionally taken a lot of resources and time to complete. As a result, many companies postpone the step at which they really dig in and start understanding and characterizing their process. Traditionally, process characterization work happens after the Phase III process has been developed. At that time, it is difficult to make significant changes.

Advances in computing power and screening designs, such as the Definitive Screening Design developed by Bradley Jones and Chris Nachtsheim, allow process optimization to happen earlier in the development life cycle. These powerful designs take up less resources and now, combined with the auto-validation method, allow for increased process understanding. A robust process can be implemented for Phase II/III studies and less effort and resources are required for late-stage process characterization activities required prior to product approval. There is a balance of spending effort earlier to save time later.

So, in the context of biopharma, before first-in-human studies, spend more time identifying the most robust cell line, rather than doing one screening study to pick the cell line that has the highest titer and moving on. In the case study I presented recently, if we had gone forward with the winner from a single round of clone selection, we would have chosen a cell line that was very sensitive to small changes in pH. Instead, after studying the cell lines, we picked the top three lines, poked at them with the parameters that we knew yielded the best product quality, and were able to identify the most stable. We could then take that cell line into our next round of early-phase development.

This kind of work is the future of bioprocessing as I see it—an integration of DOE between upstream and downstream and work. This ensures that something I'm doing upstream won't make the downstream process less robust.

BIOPHARM INTERNATIONAL: **What are important factors to consider before embarking on DOE studies?**

MCNEILL: Pre-DOE homework is very important, yet it's an often-missed step. You need to have some understanding before you do a DOE. For instance, I work in upstream and use three levels of bench-scale reactors (250 mL, 3 L, and 10 L). I need to know the

processes I am running are reproducible, whether it is January or July. I do not have control over some things that could affect the process such as ambient temperature in the room, but I need to have some understanding of the variability for not only the system I'm in control of, but also the analytics.

For example, we also need to consider the way we measure product quality. Normally, we take a small sample that is sent to the downstream team, which does a Protein A capture. Then, it is sent to the analytical department to run an assay they've developed. Note that they've developed their assay with a reference standard that has been through the entire purification process. It's very clean material. So, their understanding of the assay is based on very clean material, yet when I'm doing DOEs, I may be generating very dirty material. Thus, the analytical assay (again, developed with a very clean material) may not be as robust for product that has seen low viability cell culture conditions. If I'm only running one run from a DOE, these data may change how the modeling works if the standard deviation of the analytical assay is much greater for dirty material than it is for clean material such as the reference standard. That's why having an understanding of all the different assets you're using to make decisions is extremely important.

BIOPHARM INTERNATIONAL: **What is the difference between a screening design and a prediction design, and why would you use one over the other?**

MCNEILL: When people think of DOE, they often think about a prediction strategy and narrowing down to their top two or three parameters and spending a lot of resources understanding only these top parameters. But, I have found that sometimes this thought process is based on experience with a prior project, or lack of understanding about how powerful modern screening designs are.

So, it is important to do screening designs to figure out what is truly important for the project at hand so that critical parameter understanding is not missed. The screening design you use is also important. The definitive screening designs (DSDs) comprise three levels, so you have the opportunity to do response surface modeling with a minimal number of runs and the main effects are completely unbiased by all the second factor interactions.

You will have an understanding of what factors are important and gain prediction capability. People need to understand that certain factors may have been important for another project and cell line, but they may not be important this time. Take a step back and do that screening work, to narrow down your factors, and the auto-validation method can be used to go forward with prediction modeling to understand the design space.

BIOPHARM INTERNATIONAL: **Why choose a DSD DOE screening design over a full-factorial or central composite DOE design?**

MCNEILL: Often, the full factorial has only two

"We've even been able to model successfully with the scale-independent factors, such as starting pH or starting temperature."

levels. If you increase the number of levels for a full factorial and increase the number of factors, the number of runs becomes cost prohibitive and resource intensive. With the DSD, especially in the biology involved for upstream processes and the downstream purification, we see curvature. If you just test the high and the low, you cannot determine where the peaks and valleys are. Having three levels adds value with little cost. The only issue that I have with a DSD is that it is a very rigid design. I am a fan of the JMP statistical software platform because it offers custom design. If there is a certain combination that doesn't work, I cut that out of the design and focus on the design space that I want.

I often hear from my colleagues that they want to determine the level that needs to be on a curve. They want to do five levels instead of three. You do not need to run five different levels or settings; you can run three to determine the optimum level. I challenge them to take the high and low point, choose a midpoint, and see if they get the same result. This will reduce the number of experiments that they are doing. Another piece of advice for scientists is to stretch yourself. Go a little bit wider than you think you need to be because you want your signals to be outside the noise of the assay and be able to see where the curve is. You don't need to run five different settings of pH. You can run three, and you can determine what the optimum setting is, even if you didn't test that setting in your experiment.

If you have an OFAT (i.e., one factor at a time) study where you've done five levels, model it with only three. Take the high and the low and choose a midpoint and see if you get the same result. That can help give you confidence to reduce the number of experiments that you're doing in future projects.

BIOPHARM INTERNATIONAL: **How do the models developed from DOE using small-scale reactors perform at large scales?**
MCNEILL: We've had great success so far. We've been able to even do scale-down model qualification for the Ambr® bioreactor (Sartorius), which is great because they are so easy to set up and are very consistent from user to user. Moreover, it does not require as much media because of their small size. They are also automated, so there is less chance of variation. We've even been able to model successfully with the scale-independent factors, such as starting pH or starting temperature.

BIOPHARM INTERNATIONAL: **Is it possible to take historical experiments and perform auto-validation for small datasets method to determine if any critical factors were missed when developing the model?**
MCNEILL: Yes, you can keep adding onto previous work. As you get more data, you

keep data mining. As a small dataset starts to become medium-sized or larger, you keep updating your process understanding. Recent work with S-VEM (self-validating ensemble modeling) uses neural network and other machine learning platforms taking advantage of the fractionally weighted bootstrap and model averaging techniques. As an example, take a pH quadratic model term, and multiply it times the interaction between starting temperature and the seed density. All of those iterations are easily placed into the JMP platform. I'm excited to explore the data we've already generated and add in the manufacturing data as we get it to increase our process knowledge and understanding.

BIOPHARM INTERNATIONAL: **What are the next steps in applying machine learning to small datasets?**

MCNEILL: Do not be in an upstream and downstream silo. For instance, an upstream process that may increase yield but requires extra antifoam addition could affect the downstream process. If we can collaborate to design our early-screening designs across the groups so that the DOE is developed and designed together, all the groups will benefit.

BIOPHARM INTERNATIONAL: **Are you also collaborating with colleagues in research and discovery?**

MCNEILL: We work very closely with our cell line development group, which feeds us our candidate cell lines. They're doing a lot of screening techniques to narrow it down to the top eight-or-so candidates. We help with the initial screening designs, and we're also making sure that the cell lines that we choose are stable through multiple generations. We also work hand-in-hand with colleagues in the research group to make sure that the chosen antibody sequence is the right sequence and the most manufacturable. For me, DOE is not just something that you do late in the process. We use our DOE toolbox throughout the development process.

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Organizational Setup to Integrate Data Management, Software Development, and User Convenience from Research to GMP Production in Pharma Biotech

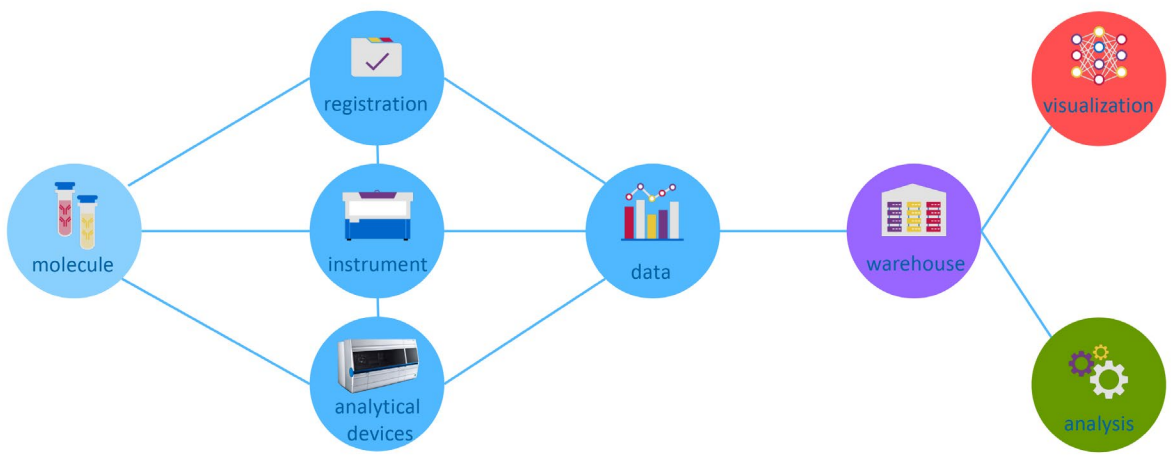
Tim Noetzel

Setup for an effective, agile, and lean organization to coordinate needs from users and IT developers.

MODERN BIOTECH PRODUCTION REQUIRES FLEXIBLE AND FAST TRANSFERS FROM RESEARCH & DEVELOPMENT TO MANUFACTURING. NOT ONLY WITHIN ONE SITE, BUT ALSO TO OTHER R&D FACILITIES AND MANUFACTURING SITES AROUND THE GLOBE TO ENSURE A CONTINUOUS SUPPLY TO THE PIPELINES, FOR THE PATIENTS. THEREFORE, TRANSFERS ARE ACCELERATED IF THE SAME ANALYTICAL DEVICES, REAGENTS, AND METHODS ARE USED. RESULTS AND ANALYSIS CAN THEN BE DIRECTLY COMPARED WITHOUT CORRECTIVE FACTORS AND FED INTO A GLOBAL CLOUD DATA COLLECTION TO WHICH ALL SITES HAVE ACCESS. THE SUCCESS OF A GLOBAL DATA COLLECTION RELIES ON THE COORDINATED OPERATION OF THE ANALYTICAL DEVICES AND AN IT MIDDLEWARE TO COLLECT, TRANSFER, AND STORE THE DATA. DATA INTEGRITY AND GOOD MANUFACTURING PRACTICE (GMP) REQUIREMENTS MUST BE FULFILLED, MASTER FILES AND RAW METADATA DEFINED, BUG FIXES AND SOFTWARE DEVELOPMENT SYNCHRONIZED.

ORGANIZATIONAL SETUP TO INTEGRATE DATA MANAGEMENT, SOFTWARE DEVELOPMENT, AND USER CONVENIENCE FROM RESEARCH TO GMP PRODUCTION IN PHARMA BIOTECH

FIGURE 1: Data in modern research labs are generated with automated, high throughput workflows using a unified, bidirectional data flow between equipment and IT systems. A modular, highly integrated system landscape is key to high-quality data. An efficient analysis of data and applying AI (artificial intelligence) algorithms can only be pursued if data apply the FAIR (Findable, Accessible, Interoperable, Re-Usable - see www.force11.org for details) data principles.

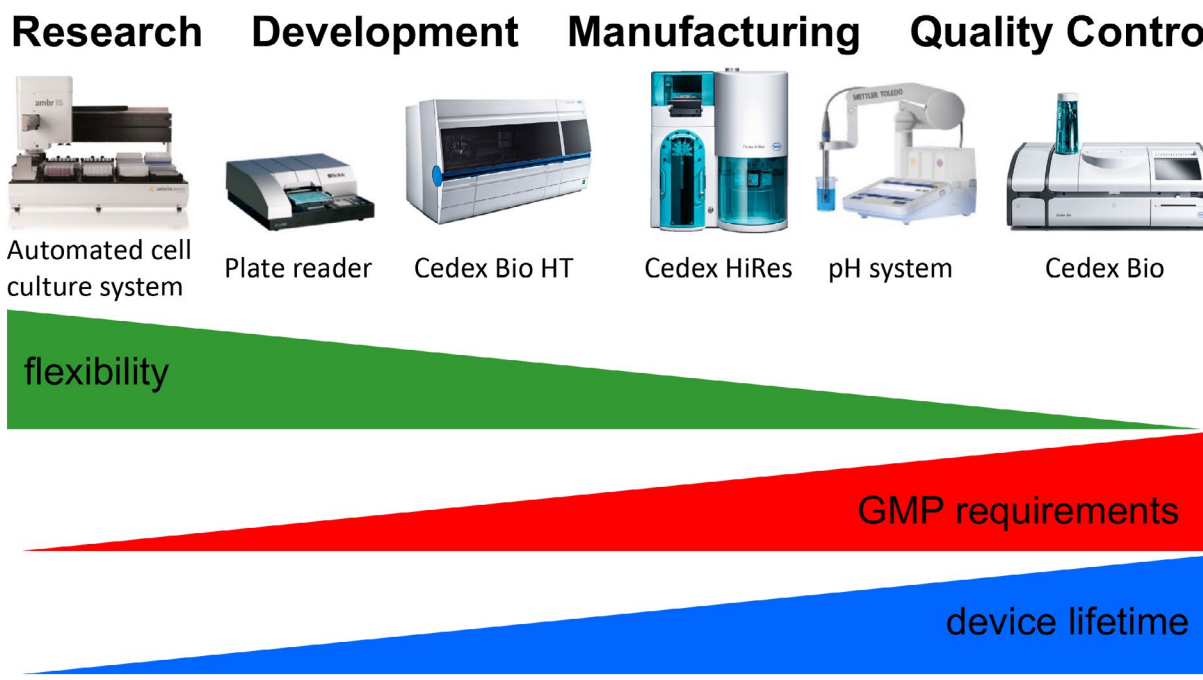


Introduction

A new, modern biotech compound starts its life in research, is further shaped and straightened in development, and finally produced and filled in manufacturing. During its product life cycle, analytical measurements as well as production metadata are stored ideally in one place to allow access of data scientists from each department. On a global scale, an effective development not only takes place within one site but in R&D facilities and manufacturing sites around the globe. Integrated data layers, automated data flows, unified master data, and seamless interlinks between labs and the digital world enable automated data workflows to ensure speed and accuracy in time to the market (FIGURE 1).

At the same time the different needs of the customers within labs across the value stream, from research to manufacturing, need to be fulfilled. We managed that by incorporating a global IT middle layer, the Sm@rtLine Data Cockpit (SDC), a flexible and agile IT solution to manage and operate devices and data (FIGURE 2). This text will not focus on the technical side of the middleware, but on the vision of a setup for an effective, agile, and lean organization to coordinate needs from users and IT developers. The model described herein is currently employed at Roche to manage data from approximately 250 devices across five global locations with roughly 2,000 lab operators.

FIGURE 2: Different customers might use the same devices but have different operational needs. Our IT SDC middleware solution is able to adapt to these individual needs and to ensure a high, unified quality of data.



Result: Living Communities of Practice (COPs)

To supply such seamless IT solutions and to be able to fulfill the requirement "valid here, valid everywhere" a combined operational excellence approach is needed to channel operational user needs, IT prerequisites and external software development cycles to a global self-organizing structure. We are developing local and global communities of practice (COPs) to coordinate new system features, data integrity requirements and life cycle bug fixes across sites (FIGURE 3).

User demands are transferred bottom to top to the decision-making meeting of IT system application managers and system owner as well as the business representatives. Local COPs decide on direct needs for their local system and setup. Regional COPs coordinate sites and prioritize bug fixes necessary for continental updates, while the global product owner board decides on future long-term developments, global systems setups, and needs according to global guidelines induced,

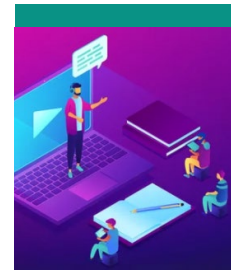
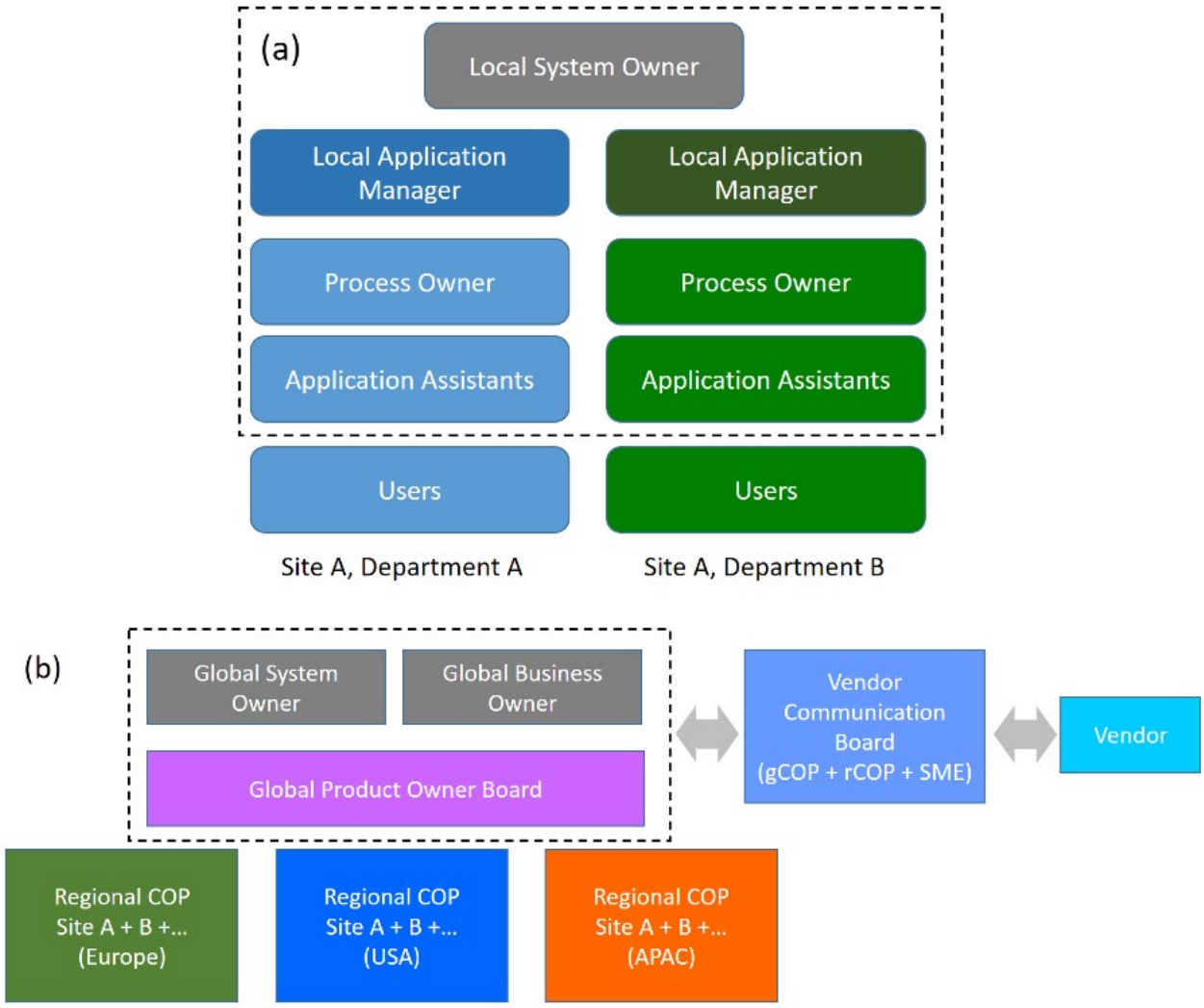


FIGURE 3: (a) The local community of practice (dotted line) coordinates in bi-monthly meeting the needs of the users. Requests are picked up by the application assistants (AA), coordinated with the process owners (PO), application managers (AM), and the local system owner (SO). (b) The local COP decides which requirements are brought to the regional and global product owner board. The global product owner board decides (dotted line) on future developments. The local SOs are responsible for technical system standards, the local POs are responsible for business (i.e., GMP) system standards. The communication with the vendor is done via the vendor communication board (VCB) to ensure one voice of the customer.



"This new way of working is more cost effective, more flexible towards the voice of the users, and enables faster decision making."

(e.g., by health authorities). Each COP applies a set of prioritization guidelines to make sure no idea is left behind. Key to a fruitful discussion across the COPs is the defined demand management and transparent decision making.

Discussions with the vendor are performed via a defined and steady vendor communication board (VCB) (FIGURE 3b). It consists of members of the global COP and the regional COPs as well as subject matter experts for the needed feature, improvement, or fix. This setup ensures one voice of the customer with the vendor and the board members are able to track and adjust long-running improvements. Budgets are regional and managed by regional COPs nevertheless the global COP manages a global budget. Therefore, even if a project is not funded from the global budget, regional improvements can still be financed if they additionally have a high prioritization ranking.

Outlook

During COP business meetings, many aspects need to be considered and balanced carefully. Large biotech production sites might have larger budgets and more users, but a smaller site might have an urgent data integrity issue threatening production. Therefore, the consent driven compromise supported by a prioritization matrix recognizing the software development cycles of the vendor supports the continuous and reliable release of new customized software.

During the release of new software, not every version has to be installed on each site, but it is the responsibility of the global COP to keep the gap between the different software versions on different sites to an acceptable minimum. Additionally to enable equal data interpretation, the COPs have to agree on data quality and format standards. Transferring raw data is easy, transferring nested GMP data and metadata is not.

This new way of working is more cost effective, more flexible towards the voice of the users, and enables faster decision making. By including experts on data integrity and compliance we have engrained a quality conception into our processes, and we continue to emphasize data excellence.

Tim Noetzel, PhD

Manager of Lab Data Solutions
Roche Pharma