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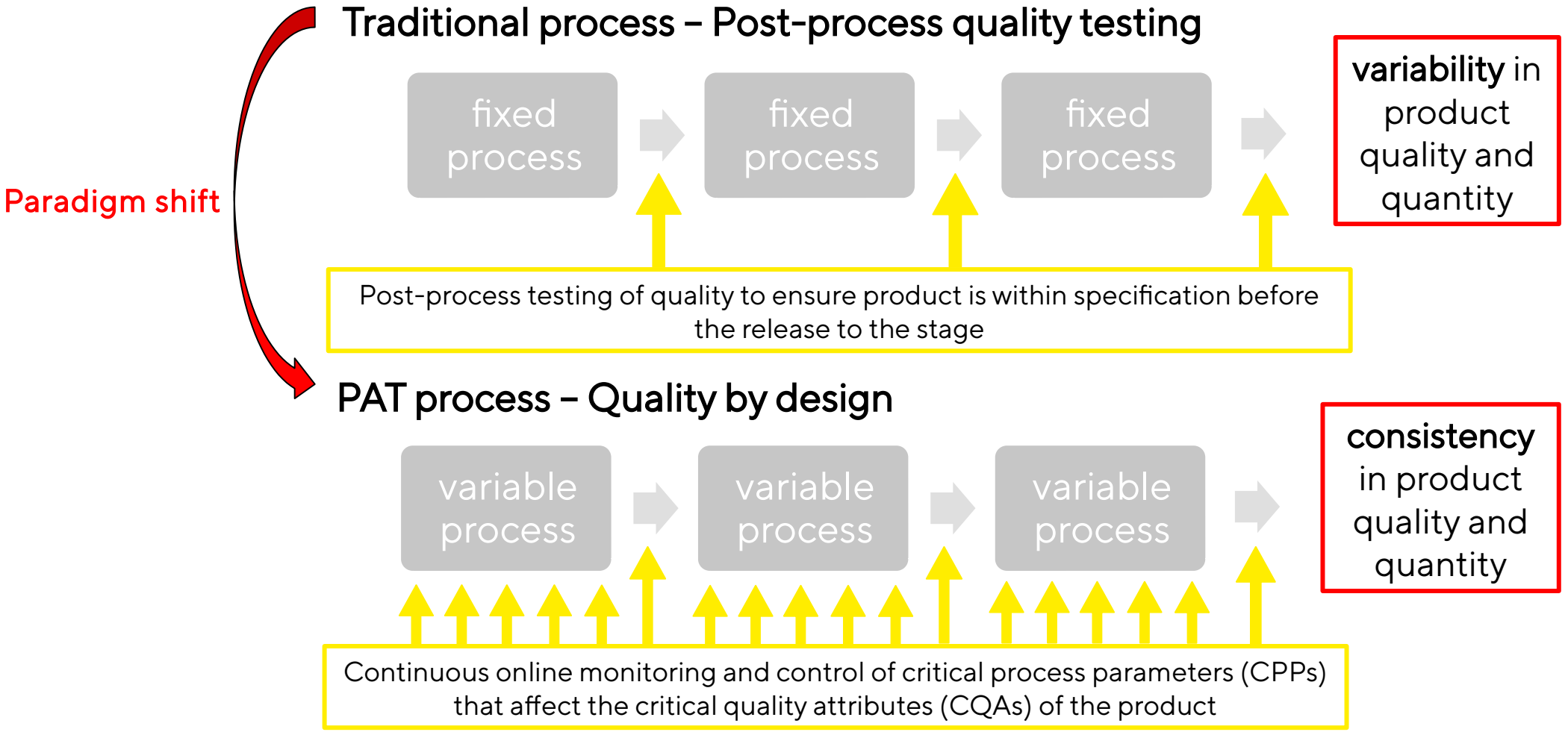
Simplifying Progress

BioPAT® Trace – Glucose and Lactate Online Monitoring and Control

Agenda

1. **Introduction to Process Analytical Technology (PAT)**
2. Working principle of BioPAT® Trace
3. Applications & process control of BioPAT® Trace

How does PAT improve a bioprocess?



Source: Svea Grieb, Kai Touw, Dan Kopec, 'What's beyond the bioprocess automation starting line', The Medicine Maker, July 2019

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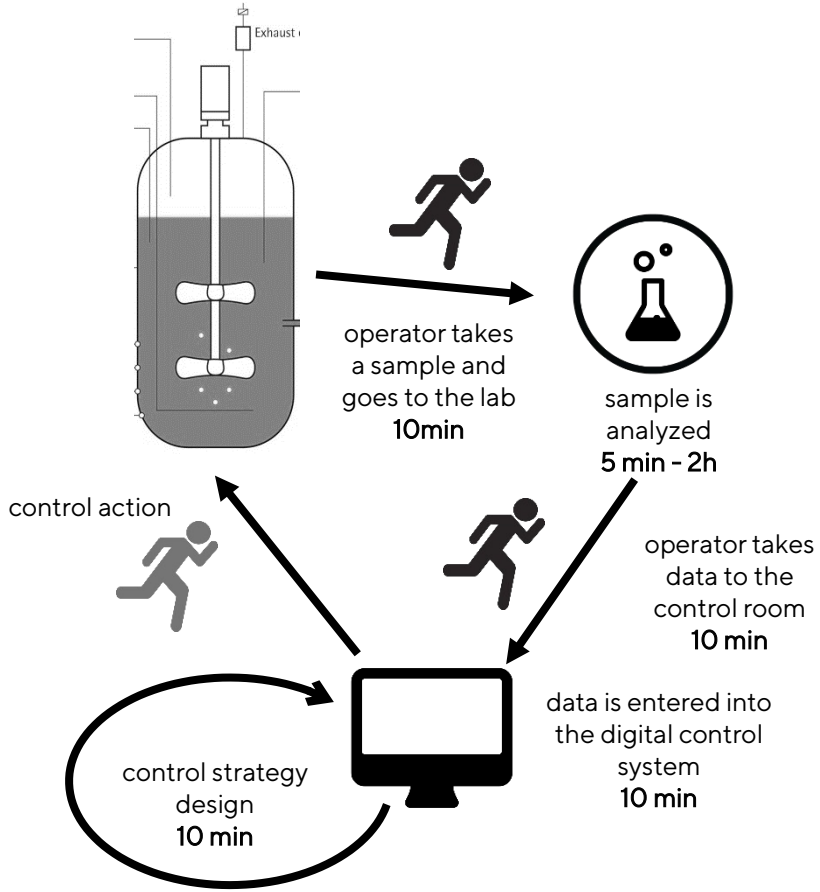
How does PAT improve a bioprocess?

Traditional process without PAT



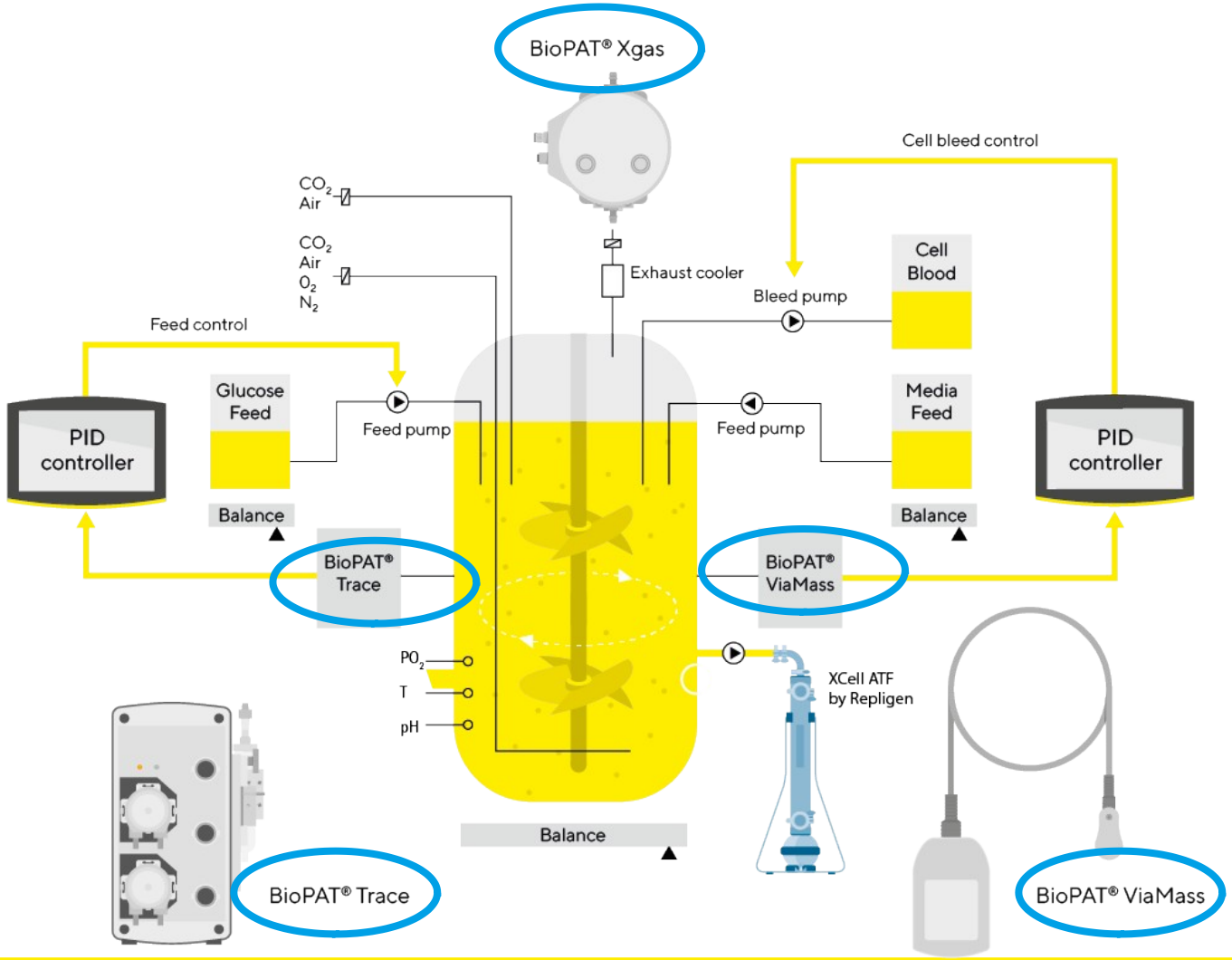
Problems / Risks

- Contamination risk through sampling
- Sample might change after removal from the reactor
- Reactor volume is lost
- Manual steps are prone to operator errors
- Up to 3h time delay between measurement and response
- No sampling over night / on weekends

Process with PAT

- ➢ Manual sampling not required
- ➢ Sample is not removed
- ➢ Reactor volume is unaffected
- ➢ Automation eliminates risk of operator errors
- ➢ Faster measurement results through data integration
- ➢ 24|7 monitoring and control

Sartorius has a comprehensive PAT portfolio



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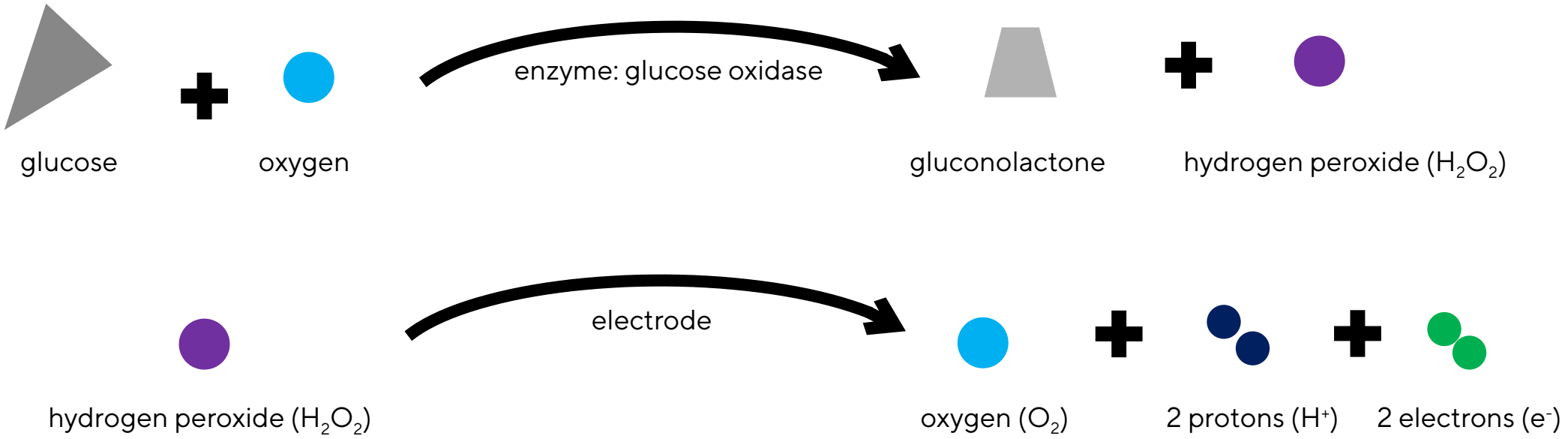
2. **Working principle of BioPAT® Trace**

3. Applications & process control of BioPAT® Trace

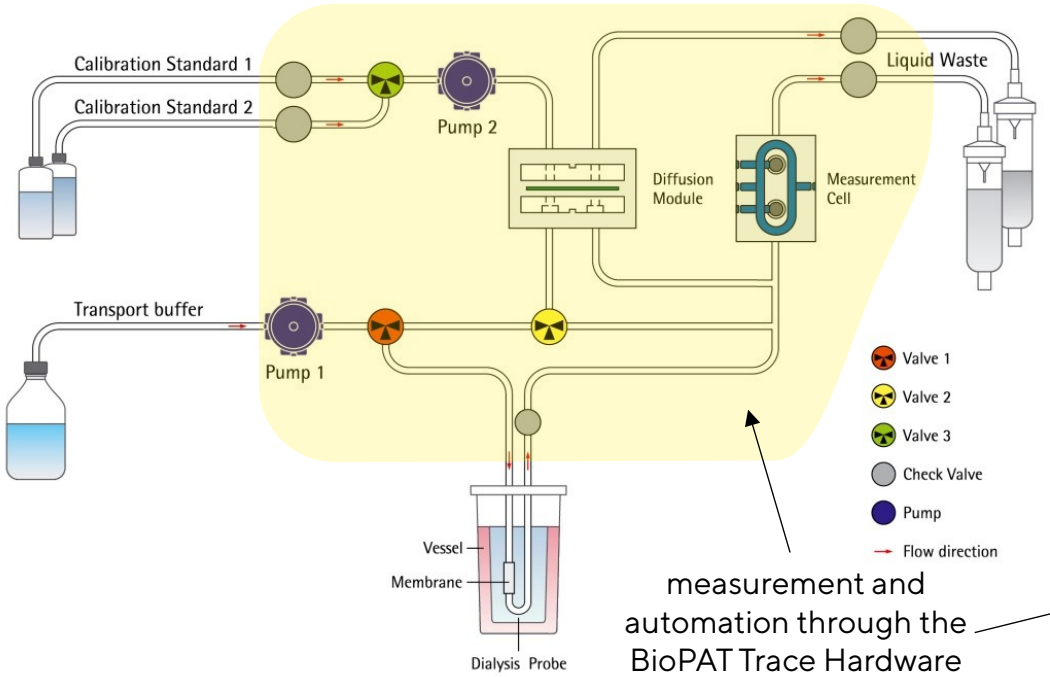
BioPAT® Trace is an automated online glucose and lactate sensor



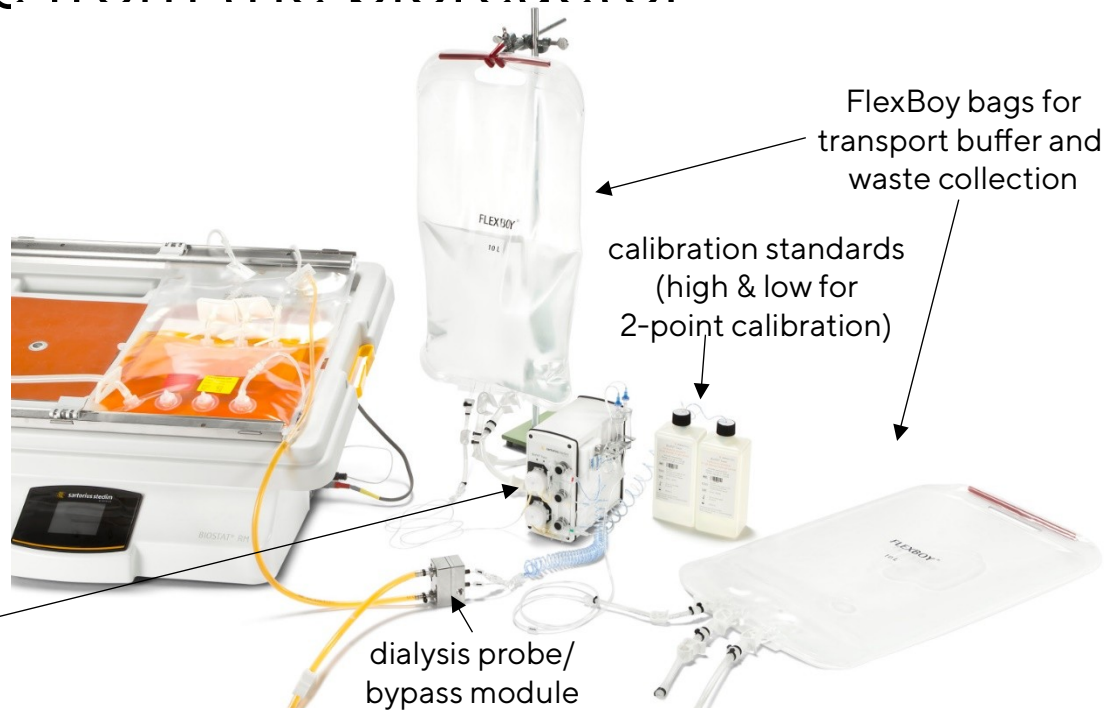
BioPAT® Trace:

- glucose and lactate biosensor based on glucose oxidase and lactate oxidase
- 1 molecule of glucose generates 2 electrons. Electrons give an amperometric signal (current).
- The measured current is proportional to the amount of glucose.

BioPAT® Trace uses a dialysis probe to measure glucose outside the bioreactor without removing a sample from the bioreactor



measurement and automation through the BioPAT Trace Hardware



FlexBoy bags for transport buffer and waste collection

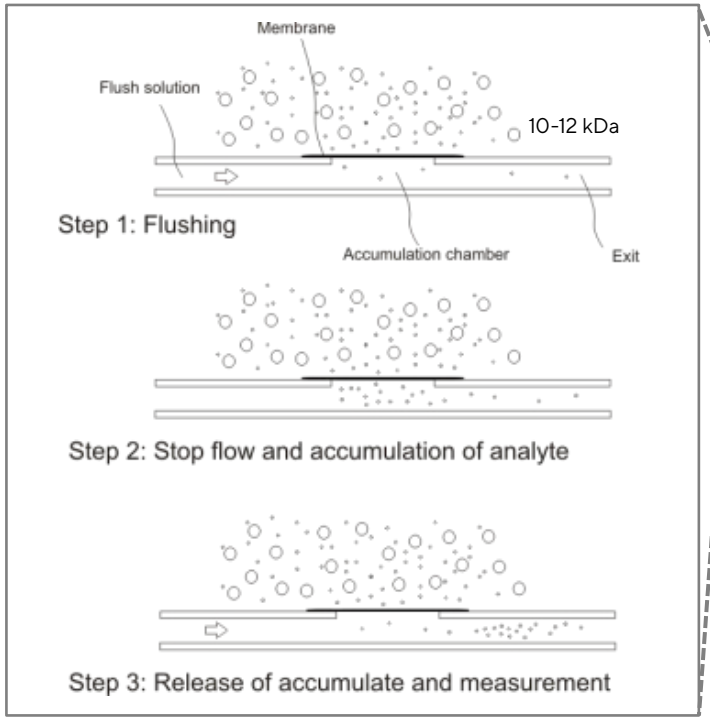
calibration standards (high & low for 2-point calibration)

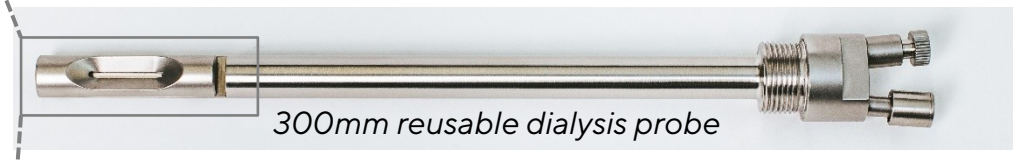
dialysis probe/ bypass module

Key features

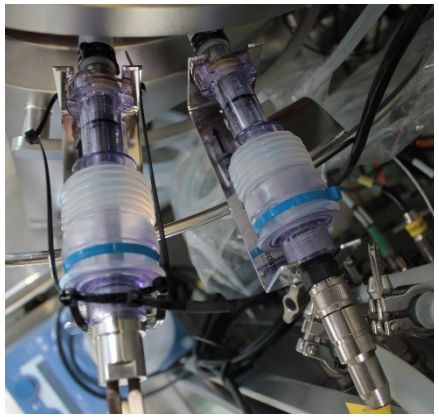
- Automatic calibration throughout the process: Several available calibration standards ensure highest resolution at your concentration of interest
- Fully closed SU system with specially designed FlexBoy bags
- SU tubeset easily removed and replaced after a run

BioPAT® Trace uses a dialysis probe to measure glucose outside the bioreactor without removing a sample from the bioreactor

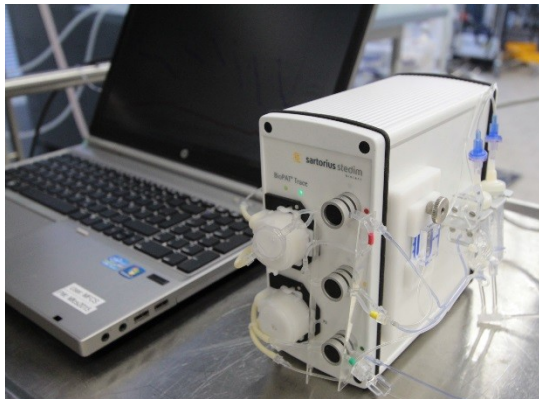




300mm reusable dialysis probe



probe inserted into the STR through KPC port



Trace software and hardware

Key technology: Trace dialysis probe

- no volume reduction
- fast response time (up to 30 measurements / hour)
- independent of media composition and viscosity

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3. **Applications & process control of BioPAT® Trace**

BioPAT® Trace can be integrated in many Sartorius bioreactors

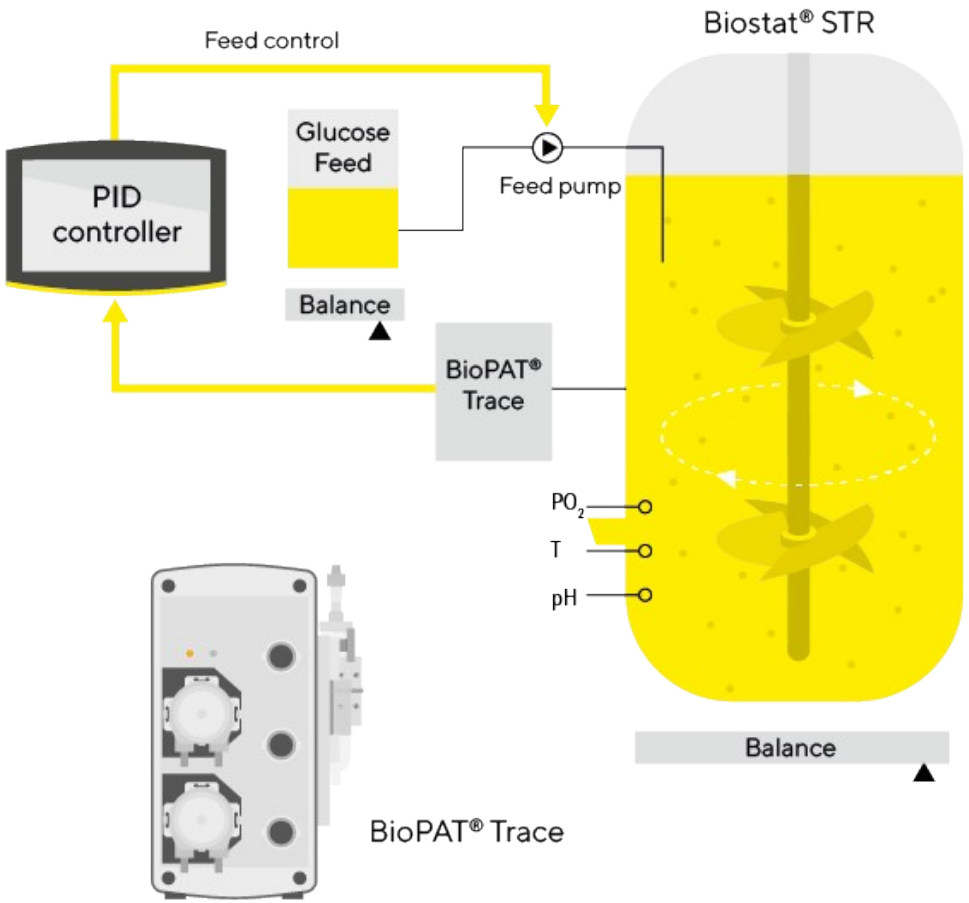
- **Biostat® STR**: multi-use probe integration via aseptic port for all sizes (50L-2000L)
- **Biostat® RM**: integration via bypass module (2L-200L)
- **Biostat® B with Univessel** (benchtop): multi-use probe integration, up to 4 parallel systems can be connected to the BioPAT® MultiTrace
- **Biostat® C and D-DCU** (stainless steel): multi-use probe integration

BioPAT® Trace in Biostat® STR

BioPAT® Trace in Biostat® RM

BioPAT® MultiTrace in Biostat® Univessel

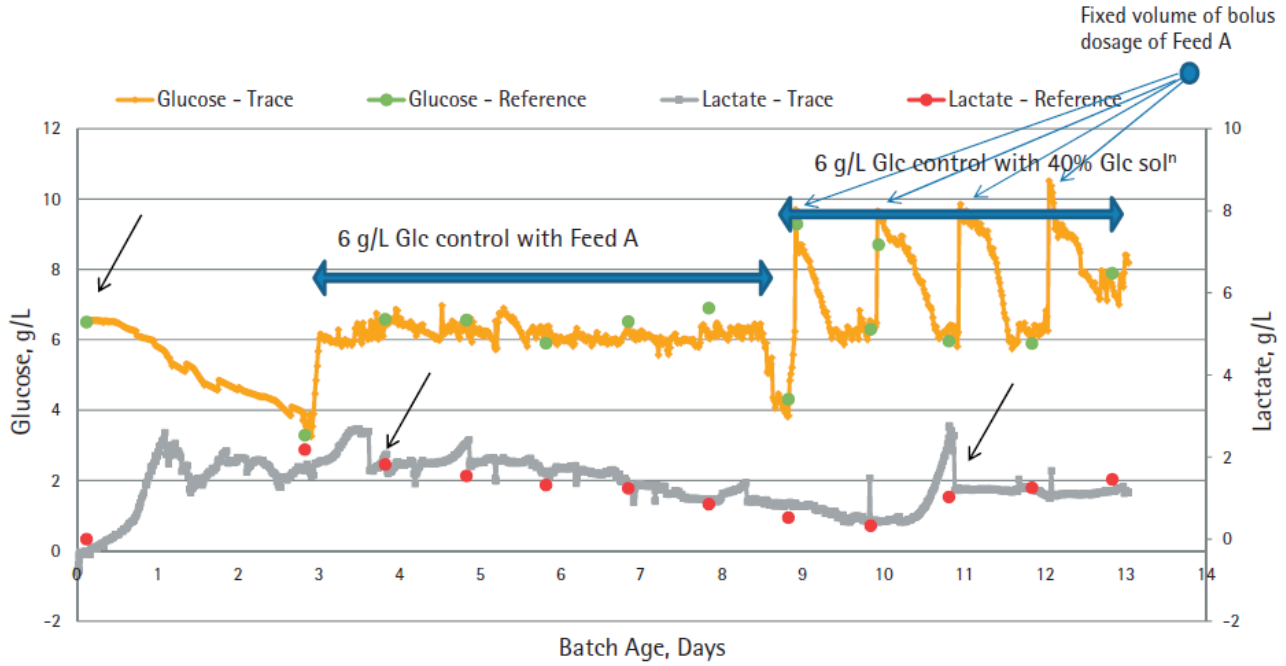
An integrated glucose/lactate sensor can automatically control glucose concentration



Automated glucose control

- prevents overfeeding and starvation of cells
- affects glycosylation and glycation
- frees up operators
- reduces risks of manual sampling

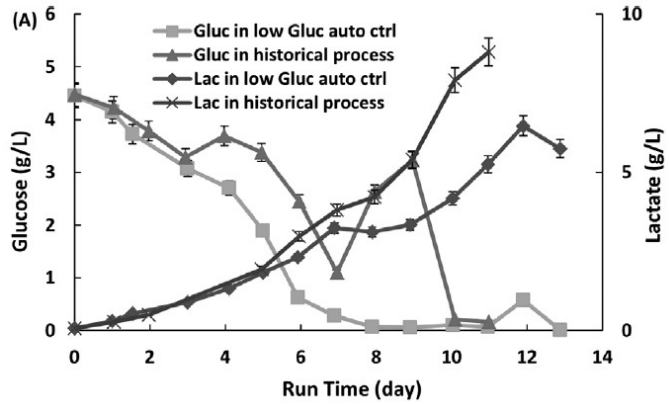
BioPAT® Trace can perform different glucose control strategies



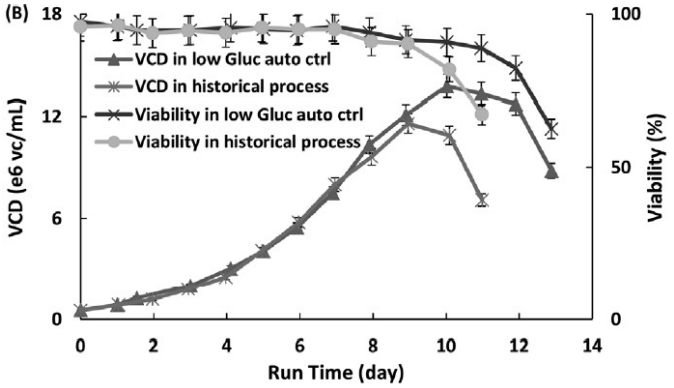
Glucose control with BioPAT® Trace:

- Consistently good correlation to offline measurement
- Can be set to continuously control the glucose level
- Can be set to trigger bolus additions

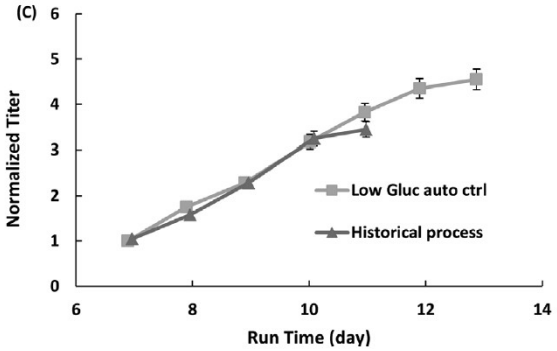
Low glucose control has significant positive impact on product titer and quality in high-lactate processes



- low glucose control (<0.5g/l) maintains low lactate levels



- low lactate levels lead to higher peak cell density and longer cell viability
- The culture time could be extended by 2 days



- low glucose control results in titer increase (32% overall titer increase)

Benefits of glucose control below 1g/l:

- longer cultivations
- higher viable cell densities
- increased product titers
- homogenious glycation

Requirements for low glucose control:

- measurement of both glucose and lactate
- high sampling frequency: min. 30min
- no sample removal required
- easy PID control implementation

Source: Advanced process monitoring and feedback control to enhance cell culture process production and robustness. Zhang et al., **Biogen**, Biotec and BioEng, 2015

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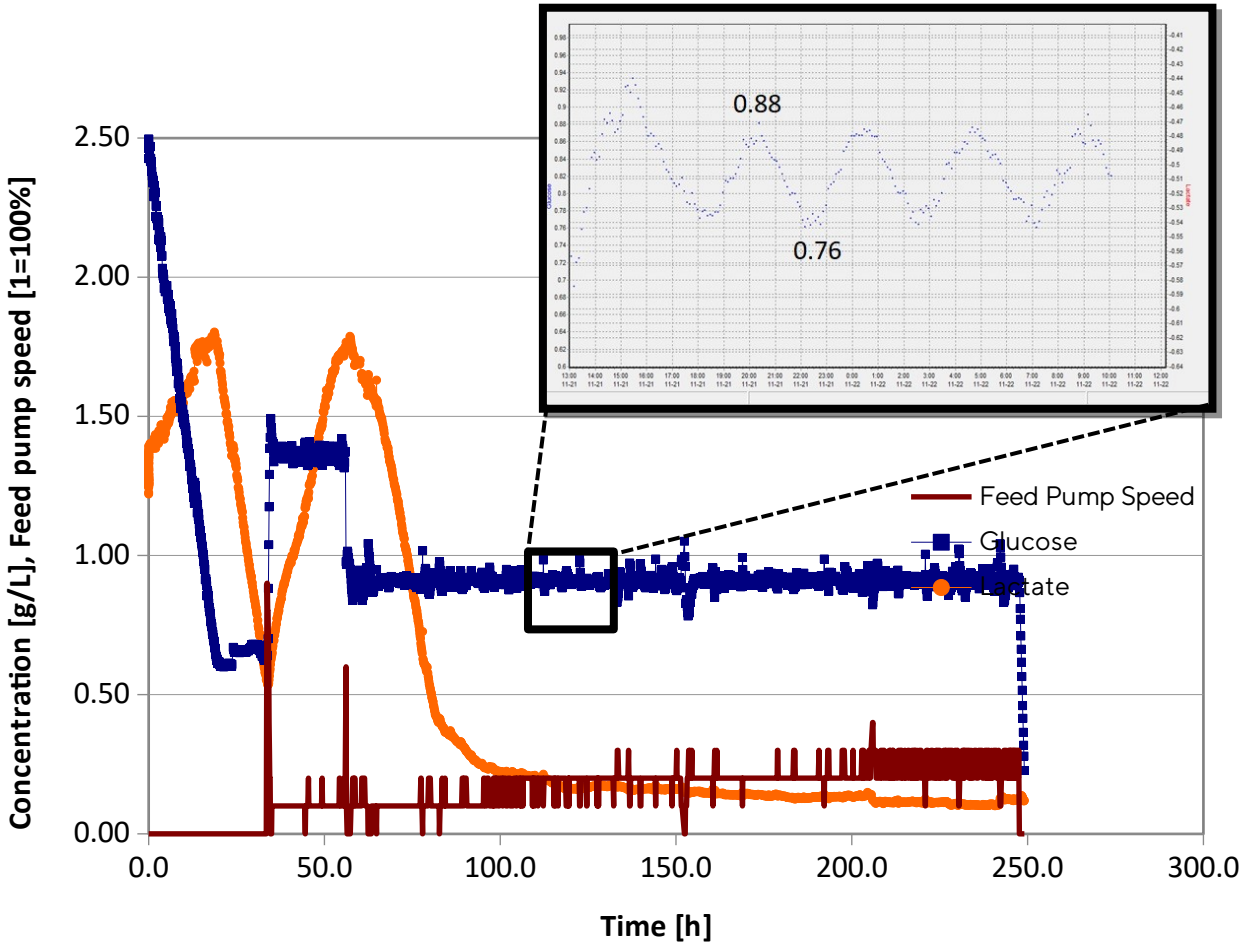
Low glucose control with BioPAT® Trace for higher process output and more consistent product quality



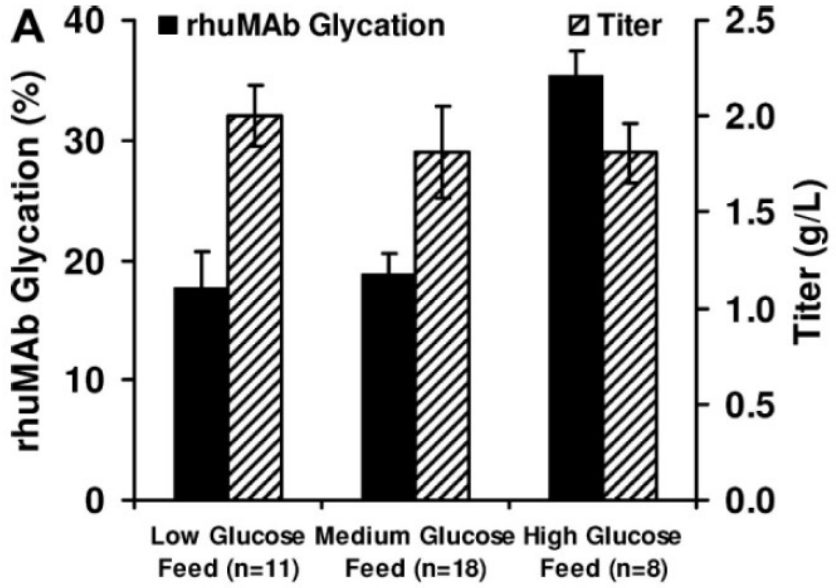
mAB process of a process controlled by BioPAT ® Trace

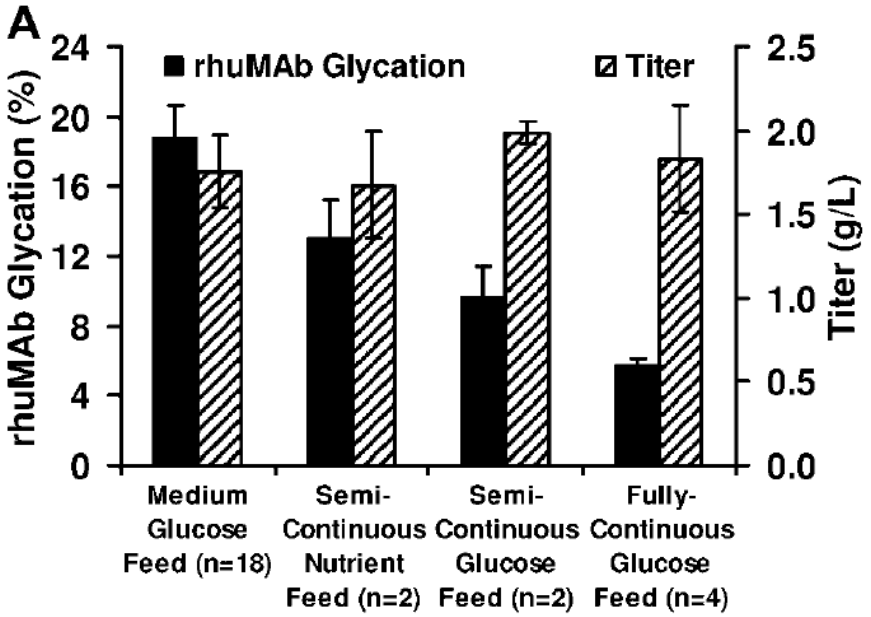
Low glucose control with BioPAT® Trace:

- near constant level control possible
- data shows that a glucose control at 1.4 g/L leads to high lactate levels, whereas 0.9 g/L glucose control can significantly reduce the lactate level
- low glucose levels lead to low lactate levels
- positive effects on peak VCD, cultivation time and titer
- narrow glucose control leads to defined glycosylation profiles of the product

Controlling glucose can reduce unwanted glycation

'...These results show that we can **control glycation** of secreted proteins by controlling the glucose concentration in the cell culture. ...'





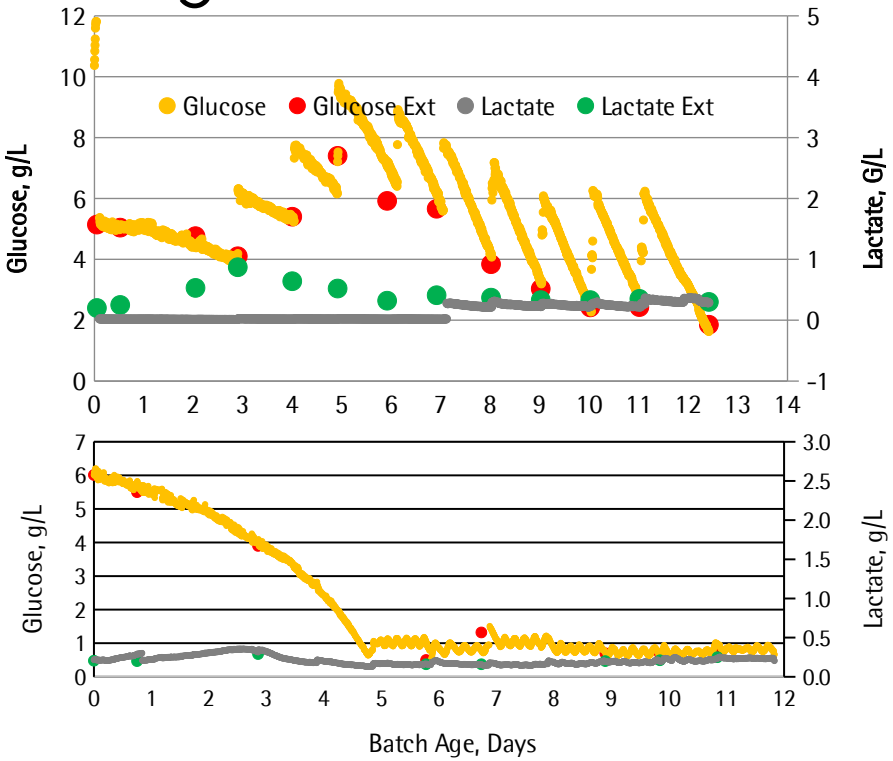
- The extent of glycation* on the monoclonal antibody rhuMAb depends on the amount of glucose increase per feed event (low = 2g/L, medium = 3g/L, high = 6g/L)
- Addition of high amounts of glucose at once (typical bolus feed) are unfavorable

- The extent of glycation depends on the feeding strategy
- The more constant the glucose concentration, the lower the extent of glycation
- Continuously keeping the glucose concentration at 3 g/L (fully continuous glc feed) gives 3-times less glycation than a bolus feed strategy (medium glucose feed)

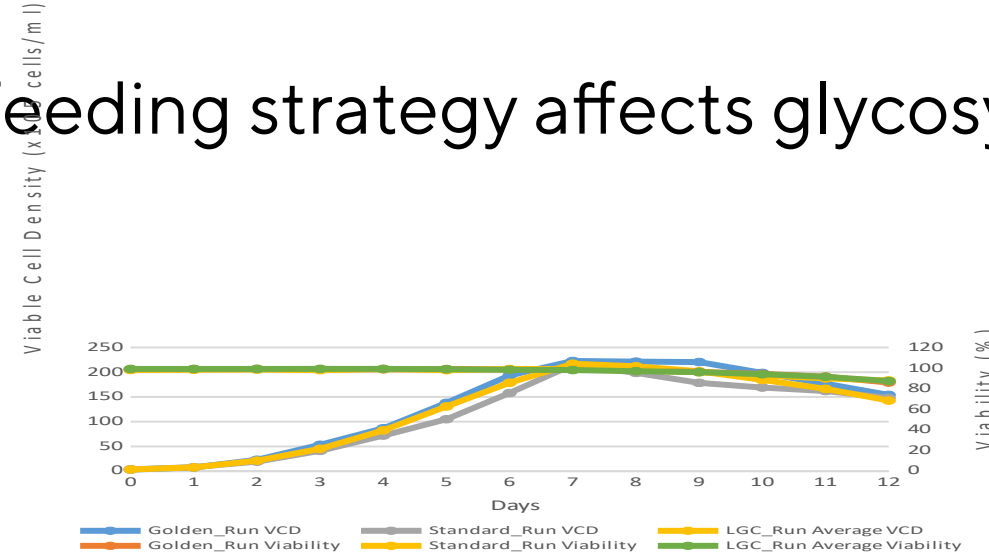
*Protein glycation is a non-enzymatic glycosylation that can occur to proteins in the human body. Glycation can also occur to recombinant antibodies during cell culture, which generates structural heterogeneity in the product and is therefore unwanted. It is different from glycosylation, which is the enzyme-mediated, site-specific addition of sugar to a protein.

Source: 'Controlling Glycation of Recombinant Antibody in Fed-Batch Cell Cultures' I.H. Yuk et al, **Genentech**, Biotec and Bioengineering, 2011

The glucose concentration & feeding strategy affects glycosylation



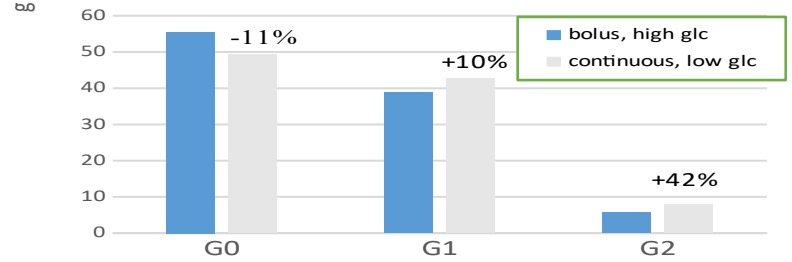
- A Cellca-2 process producing a mAB was run with the conventional time-based **bolus, high glucose** feed protocol with BioPAT Trace monitoring glucose and lactate conncentration (top graph)
- And using BioPAT Trace to **continuously control a low glucose** concentration of 0.8 g/L (bottom graph)



protein titer	
bolus	1.67 g/L
continuous	**1.74 g/L**

- The different feeding strategies did not affect the cell growth, viability or protein titer, as expected for a low lactate process, such as Cellca-2.

These results show that **we can control glycosylation** of secreted proteins by controlling the glucose concentration in the cell culture.



- The analysis of the mAB glycosylation showed a shift in the galactose glycosylation
- The proportion of monogalactosylated (G1) structures & digalactosylated structures(G2) increased significantly

Source: Internal Sartorius study, R. Anilkumar, G. Ajith, J. Lemke, S. Cheeseman, & J. Matuszczyk, Sartorius Corporate Research Department

BioPAT® Trace is widely accepted in the industry

BASF We create chemistry
Shire
Boehringer Ingelheim
Roche
gsk GlaxoSmithKline
IBA BIOTON
MSD
BIOMÉRIEUX
AstraZeneca
sanofi aventis
BAYER
St. Jude Children's Research Hospital
Finding cures. Saving children.
Fraunhofer

" I love the ease-of-use of the single-use tube set of BioPAT® Trace. It is easily set up and quickly removed after the run."
Rohan Patel
Scientist at MedImmune
MedImmune

Thank you.

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