Cell line development meets Raman spectroscopy

Success story KBI Biopharma SA develops process analysis technologies for the monitoring of diverse cell cultures and concentrations in minimal volume using Raman spectroscopy

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Challenges

Customer challenges

KBI Biopharma leads the forefront of innovation in cell line development (CLD), which is essential for producing high-quality biopharmaceuticals, such as monoclonal antibodies and vaccines.

Developing a robust and stable cell line involves extensive testing to ensure the safety and efficacy of therapeutics products, as well as the efficiency and scalability of the manufacturing process. In the context of implementing innovative process methods, KBI Biopharma has developed a fully-automated cell cultivation platform to subcultivate numerous cell cultures with more consistency.

KBI Biopharma explored the implementation of a Raman spectroscopy system for non-invasive, on-line measurement of cell concentration and viability across multiple cell cultures expressing diverse recombinant proteins. To minimize the waste of precious cell cultures, the volume of cell samples should be kept to a minimum.

Results

Summary of results

The Endress+Hauser Raman spectroscopy system, particularly the flow cell assembly, enables the measurement of a large range of cell concentrations across different protein-expressing cell lines in small volumes. The main benefits include:

- **Easy-to-use system:** Real-time data acquisition and online process provide valuable process insights with no complex modeling and an intuitive display of results
- **Production cost reduction and sustainability:** The use of minimal analysis reagents and a consumable-free design, as compared to the reference technique, helps to lower production costs. Cell counting is typically performed to monitor cell growth rates throughout the routine cell passaging process.
- **Process automation compatibility:** The Raman flow assembly allows for a seamless integration into future automated cell cultivation platforms.

“The expertise and support from the Raman application scientists at Endress+Hauser were instrumental in advancing our innovative cell line development. Their guidance helped us establish a unique model for cell counting and viability assessment of our proprietary SURE CHO-M Cell Line™. I truly valued their shared knowledge and the significant improvements they brought to our process development.”

Alexandra Martiné
Director, Cell Line Generation & Automation
KBI Biopharma

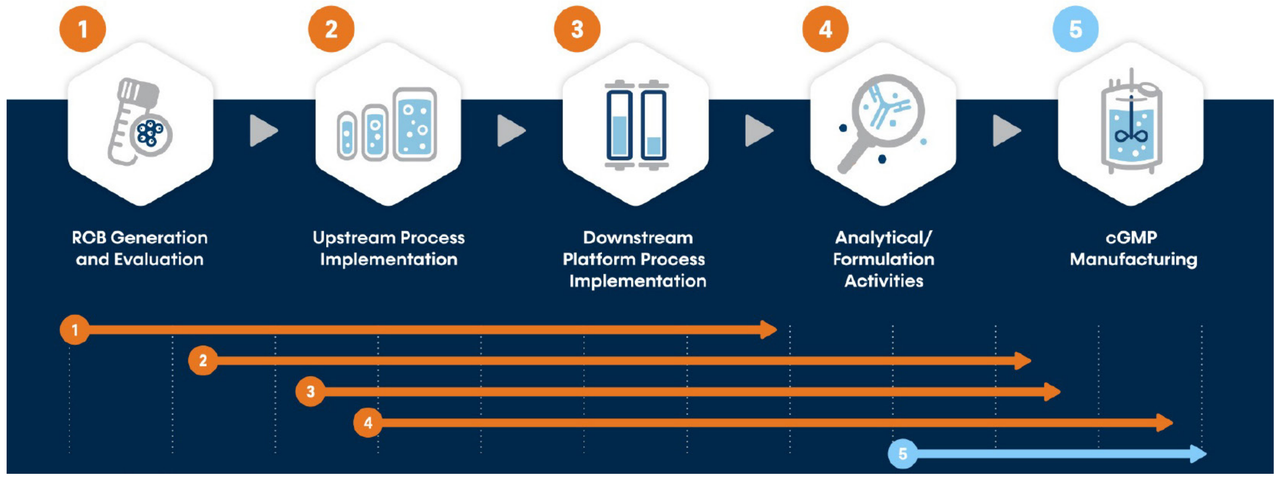
Cell line development in bioprocessing explained

CLD is a crucial step in bioprocessing to produce biological products like vaccines, antibodies, and enzymes. Such processes involve the generation of high-performance cell lines producing the desired biological product.

The process starts with selecting the cell line, from animal, human, or microbial origins, that can produce the biological product. Scientists then use producing cell lines with enhanced characteristics like productivity and stability for further process development and product formulation testing.

Once the cell line is developed, it undergoes rigorous testing to ensure the product matches with quality and activity criteria. The cell line is then used in large-scale production, where it is cultivated in bioreactors to produce the biological product in large quantities through cGMP manufacturing.

Cell line development is the first step in a development and manufacturing biotherapeutic program (image courtesy of KBI Biopharma).



KBI Biopharma's Cell Line Development team performing single cell cloning for the generation of high-performance SURE CHO-M Cell Line ™ clones

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From challenges to breakthrough in cell line development

KBI Biopharma is a world leader in developing mammalian cell lines, specifically suspension-adapted Chinese hamster ovary (CHO) cells expressing recombinant proteins. It offers a unique SUREtechnology Platform™ and specialized expertise, which is essential for turning scientific breakthroughs into life-saving medicines for patients.

KBI Biopharma focuses on enhancing its process automation at different stages of a research cell bank generation, taking into consideration key criteria:

1. **Low volume:** At early cell line development stages, volumes of cultures are within the milliliter range. Adapted fluidics must be considered.
2. **High-throughput:** Hundreds of isolated clone candidates need to be screened to select the right producer. Instrumentation capacity is crucial.
3. **Speed:** Reducing timelines in cell line development processes is critical to accelerate the biologic's development path. Achieving shortened timelines is not possible without technical innovations that speed up any cell culture assays.

Cell sample measurement using Raman spectroscopy analysis

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Our solution

KBI Biopharma chose to partner with Endress+Hauser to screen for cell growth performances during CLD using Raman spectroscopy, from the design of the experiments down to chemometrics modeling support and training.

The selected Raman system is tailored for small volume and flow paths, leveraging signal amplification and low-noise technology to deliver fast results. It consists of the following components:

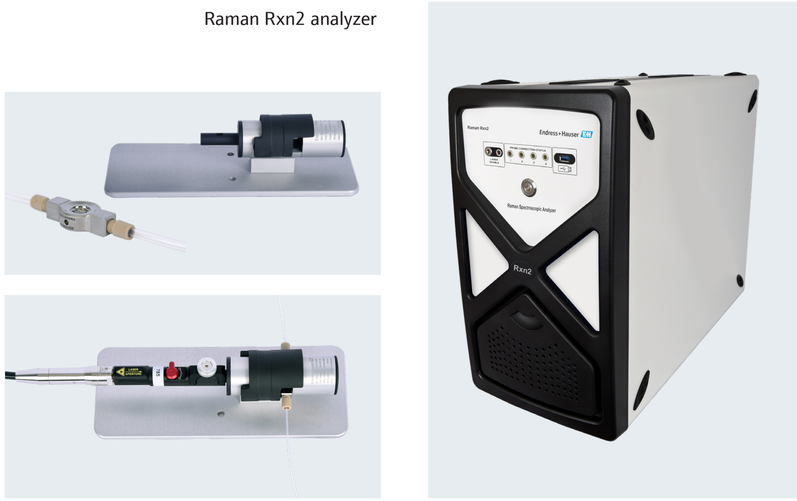
- Raman Rxn2 analyzer
- Rxn-10 probe
- Raman flow assembly

The Raman flow assembly is a specialized tool designed for biopharmaceutical labs and process development. While its primary intent is for purification and perfusion spaces, in this case it is being used for processing numerous samples in a cost-effective way for cell line engineering. The Raman flow assembly comprises:

- A reusable optic (micro flow bench) connected to an Rxn-10 probe, which has no product contact and is precisely tuned for specific flow cell and sample conditions.
- A micro flow cell that interfaces with the micro flow bench, allowing the sample to flow within it. The micro flow cell can be sterilized by approved methods and is suitable for either reuse or disposal after use.

By optimizing the process flowrate and Raman spectra acquisition parameters, KBI Biopharma minimized the volume of cell culture samples needed while maintaining top-notch Raman spectra quality.

Raman Rxn2 analyzer



(Top) micro flow cell and flow bench components; (bottom) Raman flow assembly connected to an Rxn-10 probe; (far right) Raman Rxn2 analyzer

Optimizing the installation of the Raman flow assembly and spectra acquisition with minimal volume of the cell culture

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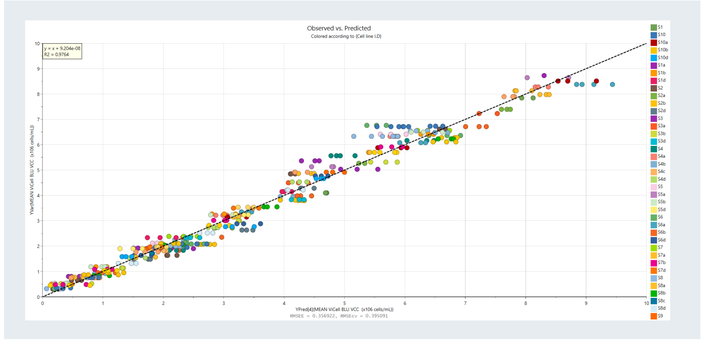
A unique Raman spectra model to predict viable cell concentration

30 SURE CHO-M Cell Lines™, each producing different types of recombinant proteins, were selected. Viable cell concentration (VCC) and cell viability were monitored using a standard reference technique to track growth performances of the cultures. Samples from each culture were then analyzed using Raman spectroscopy. Unlike the Vi-CELL BLU reference method, a Raman spectroscopy setup coupled to an automated liquid handling system eliminates the need for consumables (reagents) and allows for fully automated sampling and data collection analysis.

Data across a broad range of cell concentrations from various CHO-M expressing cell lines were acquired. Using these data, a robust chemometrics model that accurately predicts cell concentration across various productive cell lines was developed for the CHO-M cell phenotype.



The calibration curve showcases the correlation between Raman signal variations and changes in VCC. It clearly demonstrates that the predicted values closely match the actual measurements.

After applying pretreatment methods such as the first derivative and signal normalization, the Raman spectra reveal signals from samples at various VCC levels, each represented by a different color. These color variations highlight changes in the Raman signals, directly reflecting changes in the VCC content.

The corresponding score plot from the partial least squares (PLS) analysis highlights Raman spectroscopy's ability to effectively distinguish between different VCC levels. This differentiation is driven by the first component (x-axis), which captures the most significant variations in Raman signals directly linked to VCC changes. This demonstrates that the primary variation in the Raman data is due to VCC differences.

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Conclusion

The future of personalized medicine and biotherapeutics hinges on rapid and efficient CLD, driving faster drug discovery and better patient outcomes. To meet this growing demand, innovative biotechnology analytics are crucial.

With the assistance of Endress+Hauser, KBI Biopharma has successfully built a robust predictive model using a Raman analyzer system to monitor CHO-M cell cultures. This non-invasive technique, using reduced sample volumes compared to traditional Raman analysis, is suitable for cell concentration monitoring in cell line development streams.

KBI Biopharma envisions the integration of Raman technology in further upstream processing applications with extended process parameter monitoring of CHO cell cultures.

Endress+Hauser's application scientist offering training services on Raman spectroscopy data analysis

"From the initial feasibility tests to the successful rollout of the Raman spectroscopy solution, the Endress+Hauser team proved to be an invaluable partner in addressing our application needs. We were impressed by the unwavering commitment from both the Sales and Service teams, ensuring long-term support for our business."

Séverine Fagète, PhD
Vice President, Mammalian Cell Line Development
KBI Biopharma

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About KBI Biopharma SA

KBI Biopharma SA is a global contract development and manufacturing organization (CDMO) supporting the development and manufacturing of biotherapeutics.

This case study highlights Endress+Hauser's collaboration in Switzerland with KBI Biopharma, renowned for their expertise in Cell Line, Process, Analytical and Formulation Development, as well as Biomanufacturing, from preclinical up to commercial phases.

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