WHITE PAPER
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Numera® 3 System Verification

Reliability and Accuracy in Automated Bioprocess Sampling

EXECUTIVE SUMMARY

Modern bioprocess development increasingly depends on high-resolution process data. While in-line sensors provide continuous information for selected parameters, many critical process variables and quality-relevant attributes still require sample-based analysis. This creates a practical challenge: samples must be collected, prepared, transferred, and analyzed in a reproducible way without adding excessive manual workload or compromising the process.

Numera® 3 was developed as an automated bioprocess sampling platform to address this gap. The system is designed to automate the collection, handling, storage, dilution, and preparation of samples from bioreactors and connected process equipment. To assess its performance, Numera® 3 was verified against defined system requirements covering operation with and without the tape filtration unit, sample volume handling, sample waste, dilution, line length, filter tape handling, repeated filtration, system communication, and core functional behavior.

All system requirements were successfully tested and fulfilled under the defined verification conditions. Key results include:

- verified sample storage volumes of 1.5 mL, 4.0 mL, and 10.0 mL;
- operation with a 10 m sample line;
- verified dilution accuracy;
- successful filter tape alignment and retention;
- 99.9% cell removal efficiency under defined CHO test conditions;
- ≥250 filtrations with one filter tape roll;
- ≥500 filtration cycles using yeast and CHO samples;
- validated carry-over control and rinsing performance.

These results demonstrate that Numera® 3 is not only an automated liquid-handling system but also a verified platform for reliable, reproducible, and analysis-ready bioprocess sampling.

THE BIOPROCESSING CHALLENGE: RELIABLE DATA REQUIRES RELIABLE SAMPLING

Bioprocess development and manufacturing increasingly rely on frequent process measurements to improve process understanding, support Quality by Design approaches, and enable more advanced control strategies. Process Analytical Technology (PAT) has become central to this development because it supports real-time or near-real-time process monitoring and helps connect process parameters with product quality attributes [1,2].

However, many important measurements in upstream bioprocessing still depend on sample-based analytics. Viable cell density, metabolite concentrations, osmolality, product concentration, impurity profiles, and other quality-relevant attributes often require at-line or off-line analyzers. As a result, the sampling workflow itself becomes a critical part of the analytical chain.

Academic literature has repeatedly identified sampling, sample preparation, sample transfer, and data alignment as remaining gaps in bioprocess automation. Hofer et al. describe these steps as bottlenecks in otherwise increasingly automated bioprocess monitoring workflows [3]. This is especially relevant in high-throughput development, parallel bioreactor operation, and PAT-enabled workflows where frequent and consistent measurements are required.

Manual sampling can limit process insight because it is labor-intensive, operator-dependent, and restricted by working hours. It can also introduce variability in sample timing, sample volume, handling time, and preparation conditions. For advanced process monitoring and control, these sources of variability have become increasingly important.

AUTOMATED SAMPLING AS A VERIFIED SYSTEM FUNCTION

Numera®3 is designed to automate the physical sampling workflow between the bioprocess and the analytical environment. Depending on the configuration, the system can collect samples, store aliquots, dilute samples, route material to analyzers, and perform filtration for cell removal.

For such a system, reliability must be demonstrated at the platform level. Individual components may perform well in isolation, but the relevant question for users is whether the complete system performs reproducibly under defined process conditions. For this reason, the Numera® 3 verification program included both process-performance and non-process-performance requirements.

The main system verification covered requirements with and without the tape filtration unit, as well as core composition and feature requirements. The verification strategy addressed sample handling, system communication, module status, error handling, hardware indication, compatibility with defined consumables, analyzer routing functions, and system-level operation.

VERIFICATION SCOPE AND KEY RESULTS

Numera® 3 was verified against system requirements defined during the development process. All requirements were successfully tested and fulfilled under the defined verification conditions.

Key verified performance areas included:

Performance Area	Verified Result
Sample storage volume	1.5 mL, 4.0 mL, and 10.0 mL storage volumes verified
Sample line length	10 m sample line length verified
Sample waste	Defined sample waste limits verified
Dilution	Dilution accuracy requirement verified
Filtration	Operation with the Tape Filtration Unit verified
Cell removal and damage	≥99.9% cell removal efficiency under defined CHO conditions, low cell damage
Repeated filtration	≥500 filtration cycles performed under defined microbial (yeast) and CHO samples
Filter tape use	≥250 filtrations with one filter tape roll
Mechanical reliability	Filter tape alignment and secure positioning verified
Core functions	Sample routing, storage, dilution, and analyzer-related workflows verified
Carry-over	Carry-over between consecutive samples and rinsing performance were validated

These results are important because they address the complete sample pathway rather than a single isolated operation. In practice, the value of automated sampling depends on the ability to repeatedly perform the full sequence: collect the sample, move it through the system, prepare it as required, store or transfer it, and maintain the system state over repeated long-term operation.

ACCURACY AND REPRODUCIBILITY IN SAMPLE HANDLING

Accuracy in automated sampling is not limited to a single volume measurement. It includes the ability to collect the intended sample volume, limit unintended sample waste, execute dilution steps consistently, and maintain defined sample transfer behavior over realistic tubing lengths.

Sample Storage Volumes

The verification results confirm operation with different sample storage volumes, including 1.5 mL, 4.0 mL, and 10.0 mL. This is relevant because different analytical workflows require different sample volumes. A metabolite analyzer, a cell counter, a chromatographic method, and a retained sample may all have different volume requirements.

Table 1: Sample yield with various volumes of 0.9% NaCl solution (ρ = 1.005 g/mL).
The average sample volume was accurate, with a standard deviation of < 0.01 mL.

	1.5 mL	4.0 mL	10.0 mL
Sample 1	1.529	4.026	10.368
Sample 2	1.522	4.020	10.356
Sample 3	1.524	4.028	10.367
Av.	**1.525**	**4.025**	**10.364**
Std. dev.	**0.004**	**0.004**	**0.007**

Sampling with a 10m Sample Line

The verification also included an operation with a 10 m sample line. This is important for laboratory and pilot-scale environments where bioreactors, analyzers, and sampling systems are not always located directly next to each other. Longer sample lines increase the importance of controlled fluid handling, reproducible transfer, and defined cleaning or rinsing strategies. The test was performed with a buffer solution.

Table 2: Sample yield over a 10-meter sampling line for 10 mL samples of 0.9% NaCl solution (ρ = 1.005 g/mL).
The average sample volume was 10.13 g ± 0.02 g.

	Empty vial [g]	Full vial [g]	Sample volume [g]
Sample 1	11.665	21.803	10.138
Sample 2	11.720	21.823	10.103
Sample 3	11.592	21.742	10.150
Av.	**11.659**	**21.790**	**10.130**
Std. dev.			**0.020**

Dilution Accuracy

Dilution accuracy was also verified. This is particularly relevant for samples that exceed the analytical range of a connected instrument or workflows where defined dilution is needed before analysis. Inaccurate dilution would directly affect calculated analyte concentrations and could reduce confidence in downstream process decisions.

Table 3: Dilution of a 30.748 g/L Glucose solution. The results were multiplied by the dilution factor to obtain the original concentration. The average bias for dilutions up to 1:20 is <2%. The 1:100 dilution was based on a standard of 30.216 g/L Glucose; the bias was <3.5%.

Dilution	Glucose [g/L]	Bias [%]
1:2	30.161	-1.909
1:8	30.839	0.296
1:14	30.657	-0.296
1:20	31.136	1.262
1:100	31.238	3.381

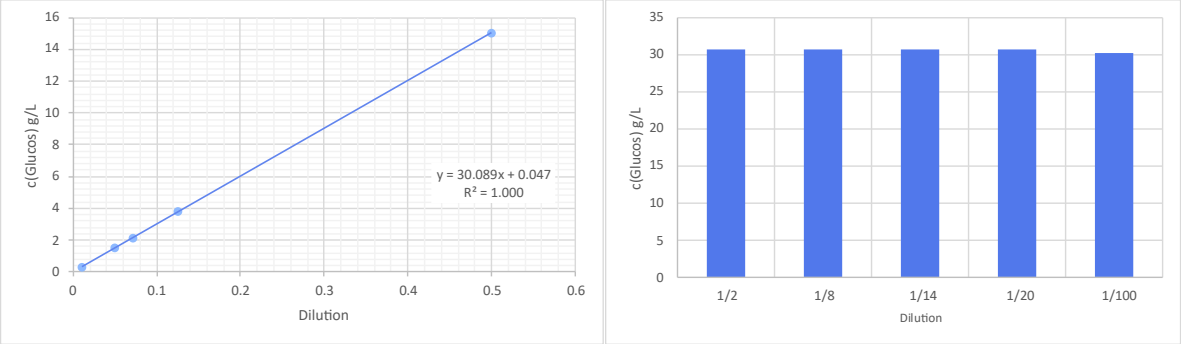


Figure 1: Dilution Accuracy: Linear regression over the dilution range of 1:2 to 1:20 and concentration bias

RELIABILITY IN FILTRATION-ENABLED WORKFLOWS

For cell-containing samples, reliable filtration is critical to preparing analysis-ready material. The Numera® 3 system verification included several filtration-related requirements, including filter tape alignment, filter tape retention, cell removal efficiency, number of filtrations per tape roll, and repeated filtration operation.

Cell Removal Efficiency

Under defined CHO test conditions, Numera® achieved 99.9% cell removal efficiency. These tests demonstrate that filtration performance was evaluated not only as a single operation, but as a repeated automated workflow under biologically relevant sample conditions.

Cell Integrity

Cell integrity (or damage) was measured during filtration tests with CHO cells at approximately 25 million cells/mL and a 2 mL loop configuration by lactate dehydrogenase (LDH) with Cobas c111. An increase in extracellular LDH concentration indicates cell lysis or membrane damage. Measuring LDH before and after filtration allows assessment of whether the filtration process adversely affects CHO cell integrity. For each sample, the LDH difference (Delta) was calculated between the processed sample and the reference sample.

$$\Delta\text{LDH} = LDH\ cc_{\ processed\ sample} - LDH\ cc_{\ refrence\ sample}$$

The results showed that membrane pore size had a significant impact on filtration performance. Smaller pore sizes, such as 0.22 µm, were more effective at reducing particulate contamination and cellular debris in the filtrate. However, they were also more prone to clogging, which limited filtrate throughput and reduced overall

volumetric yield. In contrast, larger pore sizes of up to 1.2 µm still provided effective cell retention while enabling higher filtrate volumes and improved process robustness due to reduced clogging. The trade-off was an increased presence of subcellular debris and particles in the filtrate.

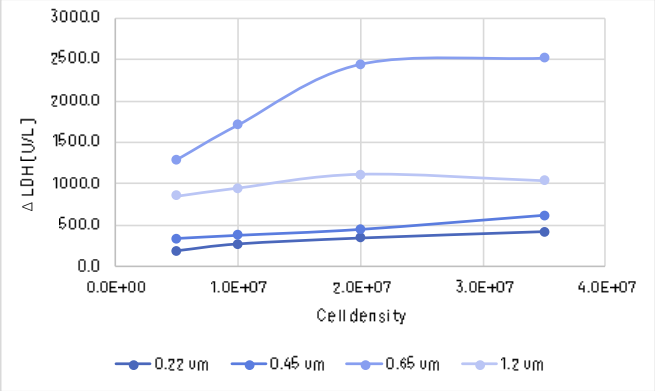


Figure 2: Cell damage measured with ΔLDH as a function of pore size and cell density.

Optimal Operating Parameters for Automated Filtration

Additional studies were conducted using CHO cell cultures with cell densities of approximately 25 million cells/mL to identify optimal operating parameters for automated filtration. Key process variables, including pump speed, transmembrane pressure, circulation time, cleaning intensity, and drying settings, were systematically evaluated. The detailed findings are presented in the white paper "Advanced Filtration in Automated Bioprocess Sampling". The study demonstrated that filtration parameters must be tailored to the specific sample characteristics, analytical requirements, and the desired balance between filtrate quality and recovery yield.

Overall, these results highlight the suitability of Numera® 3 for workflows that require automated sample preparation before downstream analytical procedures. Furthermore, the evaluation confirmed robust filtration performance not only during individual filtration events but also across repeated operating cycles, demonstrating the system's reliability for routine and long-term use.

Repeated Filtration

Repeated filtration performance was also verified under demanding test conditions. The system completed 500 filtration cycles using both high-biomass yeast samples at 50 g/L DCW and CHO samples at approximately 25 million cells/mL, with a 2 mL sample volume and 0.45 µm filter pore size. These tests demonstrate that filtration performance was evaluated not only as a single operation, but as a repeated automated workflow under biologically relevant sample conditions.

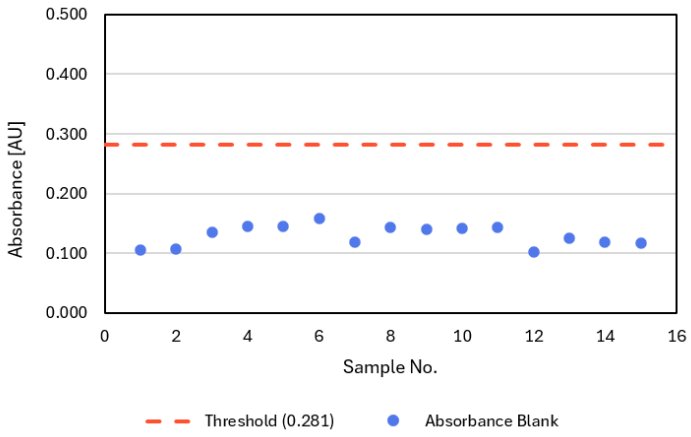
Filter tape capacity was also assessed. The system requirement of 250 filtrations with one filter tape coil was reached, and the results indicate that up to 375 filtrations can be achieved depending on the application and operating conditions.

The evaluation confirmed robust filtration performance not only during individual filtration events but also across repeated operating cycles, demonstrating the system's reliability for routine and long-term use.

VALIDATION OF CARRY-OVER CONTROL AND RINSING PERFORMANCE

Cleaning and rinsing efficiency were evaluated by sampling baker's yeast suspension (50 g/L dry cell weight, DCW) spiked with 2% bovine serum albumin (BSA), followed by a blank sample consisting of 0.1X PBS. Fifteen sample cycle draws using Numera were performed, with each cycle consisting of processing a spiked sample followed by a blank sample, allowing assessment of residual sample material within the fluid path.

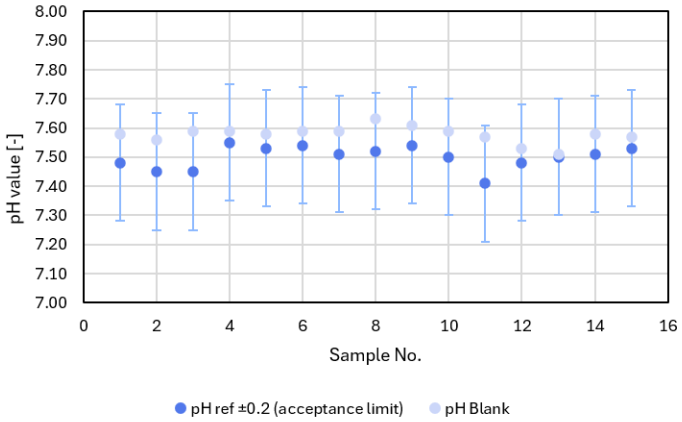
Carry-over was evaluated by measuring residual BSA in blank samples using the Pierce BCA Protein Assay. Absorbance values were compared against a predefined threshold corresponding to 1% carry-over.



No. of Samples	15
Mean	0.130 AU
Std. Deviation	0.017 AU
CV	13.3%

Figure 3: Carry-over assessment based on residual BSA in blank samples.
Blank sample absorbance values remained below the predefined threshold of 0.281, corresponding to 1% carry-over.

Rinsing efficiency was assessed by comparing the pH of blank samples with reference samples. Acceptance criteria required the pH difference to remain within ±0.2 units of the reference value.



	Reference	Blank
No. of Samples	15	15
Mean	7.50	7.58
Std. Deviation	0.04	0.03
CV	0.5%	0.4%

Figure 4: Rinsing performance based on pH comparison.
Processed blank samples remained within ±0.2 pH units of the corresponding reference samples.

RELEVANCE FOR PAT AND DIGITAL BIOPROCESSING

PAT-based bioprocessing requires more than analytical instruments. It requires a reliable connection between the biological process and the measurement system. Automated sampling contributes to this connection by reducing manual handling, improving consistency of sample timing, and enabling more frequent sample-based measurements.

Recent literature on real-time bioprocess monitoring emphasizes the need for integrated workflows that connect aseptic sampling, sample preparation, analytical measurement, and data handling [4]. Fully integrated analytical platforms, such as online HPLC or HPLC/MS systems, depend on reliable upstream sample delivery to generate timely and meaningful data [5].

In this context, Numera® 3 provides a practical automation layer between the bioreactor and the analytical environment. By automating sample collection and preparation, it supports higher measurement frequency and reduces operator-dependent variability.

SCOPE AND LIMITATIONS

The verification results presented here are based on defined Numera® 3 configurations, test conditions, sample types, and verification protocols. Performance in customer-specific applications may depend on tubing configuration, process matrix, cell type, cell density, analytical requirements, filtration settings, and cleaning strategy.

For new applications, especially those involving unusual cell types, high biomass, sensitive analytes, or specific sterility requirements, application-specific evaluation is recommended. This is consistent with good bioprocess development practice and ensures that the sampling workflow is matched to the intended analytical purpose.

CONCLUSION

Numera® 3 was successfully verified against 78 defined system requirements. The verification covered sample volumes, sample waste, line length, dilution, filtration, mechanical handling, repeated operation, and system-level functions. All requirements were successfully tested and fulfilled under the defined conditions.

For bioprocess laboratories seeking to increase data density, reduce manual workload, and improve reproducibility, these results provide a strong technical basis for using Numera 3 as an automated sampling platform. By combining reliable liquid handling, verified filtration functions, and defined system behavior, Numera 3 supports the transition from manual sampling toward automated, data-rich bioprocess development.

CONTACT

The results presented in this white paper are part of Securecell's broader validation and verification activities for the Numera® automated bioprocess sampling system. Numera is designed to support reliable, automated sampling, sample preparation, and integration with analytical workflows in modern bioprocess development.

To learn more about Numera and its role in automated bioprocess sampling, visit: www.securecell.ch/numera

For application-specific questions, technical discussions, or to evaluate how Numera could support your bioprocess workflow, please contact the Securecell sales team: www.securecell.ch/contact

Our team will be happy to discuss your process requirements, sampling strategy, analytical setup, and suitable Numera configuration.

Securecell AG, In der Luberzen 29, 8902 Urdorf, Switzerland

References

[1] Hofer et al., 2020, *A Reliable Automated Sampling System for On-Line and Real-Time Monitoring of CHO Cultures,*
[2] Carvalho et al., 2025, *A Review on Quantitative Process Analytical Technology for Continuous Downstream Processing of Monoclonal Antibodies*
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[5] Liu et al., 2022, *A Fully Integrated Online Platform For Real-Time Monitoring Of Multiple Product Quality Attributes In Biopharmaceutical Processes For Monoclonal Antibody Therapeutics*