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# Cell Line Development

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# In This Guide

**Leverage a partner with an extensive portfolio and accelerate your Cell Line Development.**

### AUTOMATION SOLUTIONS

for those looking to Increased demand for higher productivity and increased capacity

### ANALYTICAL SOLUTIONS

For those looking to achieve higher expression levels

### DATA SOLUTIONS

For those looking to Increase scalability

## WHY IT MATTERS

From producing cutting-edge vaccines to developing antibody drug conjugates to treat cancer, the use of cell lines is changing critical facets of the biopharma industry.

Cell line development (CLD) is no simple task. Putting a lifesaving biologic, such as a targeted monoclonal antibody, on the market requires years of drug discovery research, development, clinical trials and scaling up for commercialization. Establishing cell lines is a long process, with numerous steps and challenges.

Our Danaher Life Sciences companies support biopharma and service providers in their endeavor to accelerate the CLD process with its comprehensive platform of best-in-class science and technology companies. We recognize the necessity for solutions that eliminate bottlenecks faster and aim to address this with our extensive instrument, software and services portfolio.

Enabled by

# Analytics

Gain critical insights faster with scalable solutions

APPLICATION NOTE

# High-throughput IgG quantitation platform for clone screening during drug discovery and development

**Cathy Olsen, PhD | Sr. Applications Scientist | Molecular Devices**
**Hannah Byrne, PhD | Head of Biological Sciences | ValitaCell**

## Introduction

Biologic drugs are the largest and fastest growing segment of the pharmaceutical industry with sales of €500bn and an annual growth of 8%. Every manufacturing process for potential biologics begins with cell line development, whether it's for clinical trials or a market launch. Monoclonal antibodies (mAb) have established themselves as the leading biopharmaceutical therapeutic modality. The establishment of robust manufacturing platforms is key for antibody drug discovery efforts to seamlessly translate into clinical and commercial successes. The accurate and reliable measurement of mAb (e.g., IgG) titer is essential in the development and subsequent manufacture to ensure optimal cell culture performance for the production of all biologics (Figure 1). The ability to reliably monitor protein titer in real time throughout a bioprocess allows operators to rapidly adjust the process conditions for maximum protein output while minimizing process time. Quick access to titer data also enables earlier decisions regarding preparation of downstream processes, further reducing the production timeline.

### Benefits

- Fast, homogeneous, and automation-friendly assay with results for 96 samples in less than 15 minutes
- Low sample volume and limited test sample pre-preparation, allowing analysis of samples in a crude matrix
- Precise measurement of IgG from 2.5 to 2000 mg/L

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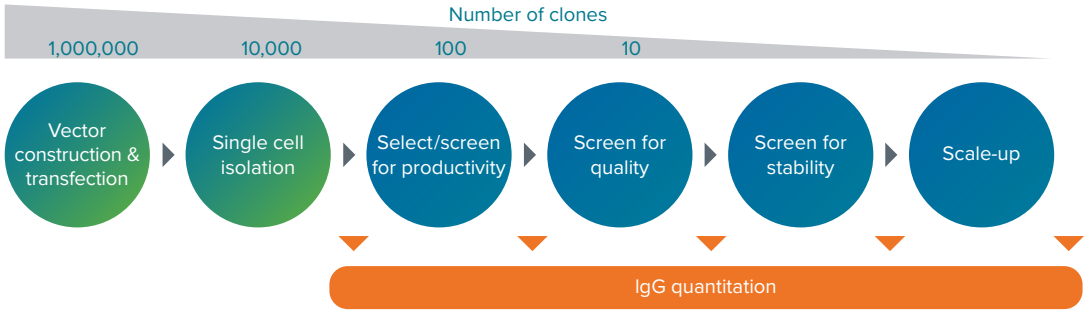


***Figure 1.*** *The cell line development process from cell transfection to scale-up. The cell line generation process is highly complex, tedious, and time consuming as clones with high productivity, stable long-term expression and good product quality are rare occurrences. Hence, screening strategies are implemented at all stages of biologic drug production. The slowest step in developing a new mAb with therapeutic potential is clone selection, which is hindered today by legacy screening technologies. Additionally, current workflows are single function, costly, and require specialized training.*

Of the various technologies currently employed by the biopharmaceutical industry to quantify mAbs, the gold standard Protein A HPLC, bio-layer interferometry, enzyme-linked immunosorbent assay (ELISA), and immunoturbidimetric assays are common methods. They all have distinct features including cost per test, cost of hardware, and experience of staff required to execute the experiment. Importantly, some of these techniques require various steps to prepare the samples for analysis, such as centrifugation or dilution to remove whole cells, cellular debris, and contaminants. Despite their widespread adoption in industry, the high cost (Protein A HPLC), sensitivity to cellular contamination leading to variability in results, susceptibility to human error, labor intensive workflow (ELISA), and slow time-to-result (>3 hours in some cases) remain as big hurdles for users looking to adopt Protein A HPLC and ELISA throughout their bioprocessing workflows for the quantitation of IgG.

Here we provide an overview of a fully optimized rapid, robust, and accurate IgG titer platform combining ValitaCell's ValitaTiter IgG quantitation assay with a suite of Molecular Devices fluorescence polarization (FP) configured microplate readers. The ValitaTiter assay range measures IgG concentrations from 2.5 to 100 mg/L or 100 mg/L to 2000 mg/L, with a simple add-and-read protocol. ValitaTiter plates come precoated with a fluorescently-labeled, target-specific probe that the user reconstitutes prior to IgG test sample addition. The assay is performed in less than 15 minutes and can be incorporated into the bioprocess workflow in a 96- or 384-well plate format. The assays are high throughput and can be fully automated. Analysis can be carried out in crude cell culture media containing up to 10 x $10^6$ cells/mL with a low sample volume and limited test sample pre-preparation. Assay detection can be performed using fluorescence polarization on Molecular Devices microplate readers: SpectraMax® iD5, i3x, Paradigm®, and M5 Multi-Mode Microplate Readers. (The i3x and Paradigm readers require the Fluorescence Polarization Detection Cartridge.)

The SpectraMax Multi-Mode Microplate Readers provide excellent flexibility, and most include absorbance, fluorescence, and luminescence with configurable options for fluorescence polarization (FP), time-resolved fluorescence (TRF), and FRET. Upgradeable modules are also available including western blot, cell imaging, and injectors for fast kinetics.

| | ValitaTiter | Surface interferometry | ELISA | HPLC |
|---|---|---|---|---|
| Total assay time [96 samples] | <15 min; <30 min | 55–65 min | 6+ hours | 25–45 hours |
| Sample volume [µL] | 5–30 | 180+ | 100 | 1000–2000 |
| Measurement range [mg/L] | 2.5–100; 100–2000 | 0.025–2000 | 0.5–5 | >10 |
| Precision | <2mP | <5% | <5–10% | <2% |
| Robust to cell contamination | 10 x $10^6$ million cells/mL | Cells removed prior to analysis | Cells removed prior to analysis | Cells removed prior to analysis |
| Automation friendly | Yes | No | Yes | No |

***Table 1.*** *Overview of key features of ValitaTiter versus competitors.*

## Assay principle

ValitaTiter and ValitaTiter Plus are rapid, high-throughput assays quantifying IgG-Fc interactions with a fluorescently labeled derivative of protein G using FP for detection. FP effectively analyzes changes in the size of molecules (Figure 2). “Fixed” fluorophores are excited by polarized light and preferentially emit light in the same plane of polarization. The rotation of the molecules between absorption and emission of the photon results in “twisting” the polarization of the light. Small molecules tumble faster in solution than larger molecules. Hence, the change in molecule size upon the binding of a fluorescently labeled Fc-specific probe can be detected using the degree of light depolarization. When the fluorescently labeled IgG-binding peptide is unbound, it tumbles rapidly, depolarizing the light more than when bound to an IgG (which is ~20 times larger). The detection of FP involves excitation of the solution with plane polarized light and subsequent measurement of emitted light intensity in both the parallel (polarized portion) and perpendicular (depolarized portion) planes to the exciting light. The FP is expressed as a normalized difference of the two intensities, typically expressed in millipolarization units (mP).

# Materials

- ValitaCell ValitaTiter Kit (cat. #VAL003)
- ValitaCell ValitaTiter Plus Kit (cat. #VAL004)
- Sigma IgG standard (Sigma cat. #I2511)
- XP Media™ CHO Growth A (Molecular Devices cat. #K8860), supplemented with 4 mM L-glutamine
- SpectraMax iD5 Multi-Mode Microplate Reader
  - Fluorescence Polarization Filters Polarized Vertical and Horizontal 400nm–750nm (Molecular Devices cat. #6590-0129 and 6590-0130, provided with the reader)
  - Set of 2 Fluorescence Polarization Filters 535nm BW 25nm Polarized Vertical & Horizontal (Molecular Devices cat. #6590-0137)
- SpectraMax i3x Multi-Mode Microplate Reader
  - Fluorescence Polarization (FP-FLUO) Detection Cartridge (Molecular Devices cat. #0200-7009
- SpectraMax Paradigm Multi-Mode Microplate Reader
  - Fluorescence Polarization (FP-FLUO) Detection Cartridge (Molecular Devices cat. #0200-7009)
- SpectraMax M5 Multi-Mode Microplate Reader

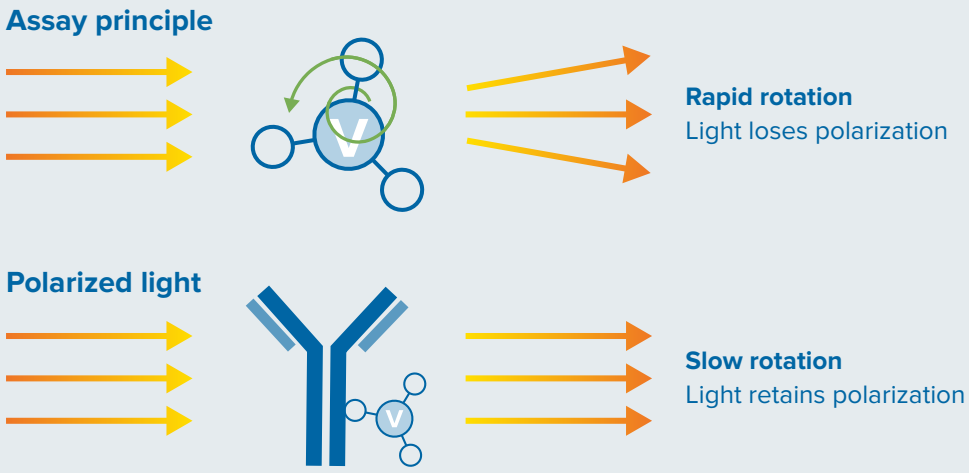


***Figure 2.*** *The assay applies fluorescence polarization to quantify Fc-containing IgG. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).*

# Method

1. A serial dilution of IgG standards was performed, using XP Media/L-glutamine as the diluent, to concentrations from 2.5–100mg/L (ValitaTiter) or 100–2000mg/L (ValitaTiter Plus).
2. 60 µL of medium was pipetted into each well of the ValitaTiter or ValitaTiter Plus plate to reconstitute the probe.
3. 60 µL of prepared standards were then added to the appropriate wells.
4. Well contents were mixed by gently pipetting up and down three times (see Figure 3 for assay workflow overview).
5. Assay plates were incubated in the dark for five minutes (ValitaTiter) or 15 minutes (ValitaTiter Plus) at room temperature prior to measurement on a suite of Molecular Devices microplate readers, using the identified fully optimized methods outlined in Table 1 (ValitaTiter) and Table 2 (ValitaTiter Plus).

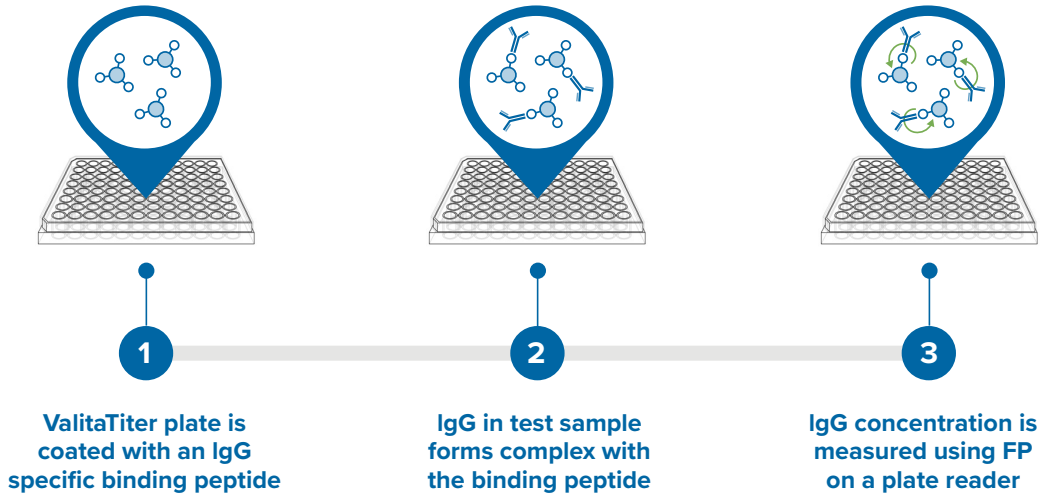


***Figure 3.*** *Each well of the assay plate is precoated with a fluorescently labeled Fc-specific probe (1). An IgG sample binds to the probe (2). Binding is measured via fluorescence polarization (3).*

| Setting | iD5 | i3x | M5 | Paradigm |
|---|---|---|---|---|
| Measurement mode | Fluorescence Polarization | Fluorescence Polarization | Fluorescence Polarization | Fluorescence Polarization |
| Excitation | 485 nm monochromator | 485 nm | 485 nm | 485 nm |
| Emission | 535 nm filter | 535 nm | 525 nm, 515 nm cutoff | 535 nm |
| PMT gain | Low | --- | Medium | --- |
| G Factor | 1.0 | 1.0 | 1.0 | 1.0 |
| Attenuation | None | --- | --- | --- |
| Integration time or flashes | 400 ms | 400 ms | 100 flashes/read | 400 ms |
| Read height (mm) | 3.6 | 4.8 | --- | 4.1 |
| Settling time (ms) | --- | --- | 100 | --- |

***Table 2.*** *Optimal instrument settings for ValitaTiter assay Fluorescence Polarization measurement on Molecular Devices microplate readers. SpectraMax i3x and Paradigm readers require the FP-FLUO detection cartridge. Settings not required by a reader are indicated by '---'.*

| Setting | iD5 | i3x | M5 | Paradigm |
|---|---|---|---|---|
| Measurement mode | Fluorescence Polarization | Fluorescence Polarization | Fluorescence Polarization | Fluorescence Polarization |
| Excitation | 485 nm monochromator | 485 nm | 485 nm | 485 nm |
| Emission | 535 nm filter | 535 nm | 525 nm, 515 nm cutoff | 535 nm |
| PMT gain | Low | --- | Low | --- |
| G Factor | 1.0 | 1.0 | 1.0 | 1.0 |
| Attenuation | 1 OD | --- | --- | --- |
| Integration time or flashes | 400 ms | 400 ms | 100 flashes/read | 400 ms |
| Read height (mm) | 5.5 | 5.2 | --- | 4.8 |
| Settling time (ms) | --- | --- | 100 | --- |

***Table 3.*** *Optimal instrument settings for ValitaTiter Plus assay Fluorescence Polarization measurement on Molecular Devices microplate readers. SpectraMax i3x and Paradigm readers require the FP-FLUO detection cartridge. Settings not required by a reader are indicated by '---'.*

# Results

An investigation was carried out in order to identify the optimal parameters for using ValitaCell's ValitaTiter assays on Molecular Devices suite of multi-mode microplate readers in order to provide a cost-effective, high-throughput IgG quantitation platform for use in high-throughput drug discovery and development. IgG standard curves were prepared and analyzed using a simple add-and-read method, with no sample or plate pre-preparation or wash steps required, and an easy workflow.

Superior results were obtained using built-in monochromator optics for excitation at 485 nm and a 535 nm FP filter set for emission using the SpectraMax iD5 reader. IgG Standards from 2.5mg/L to 100mg/L (ValitaTiter) or 100mg/L to 2000mg/L (ValitaTiter Plus) were detected with a high degree of linearity ($R^2$=0.99) across the entire range (Figure 4). Comparable data for ValitaTiter (Table 4) and ValitaTiter Plus assay (Table 5) were obtained with the SpectraMax i3x, Paradigm, and M5 readers. A preconfigured protocol in SoftMax® Pro Software automated the mP calculations and curve plotting.

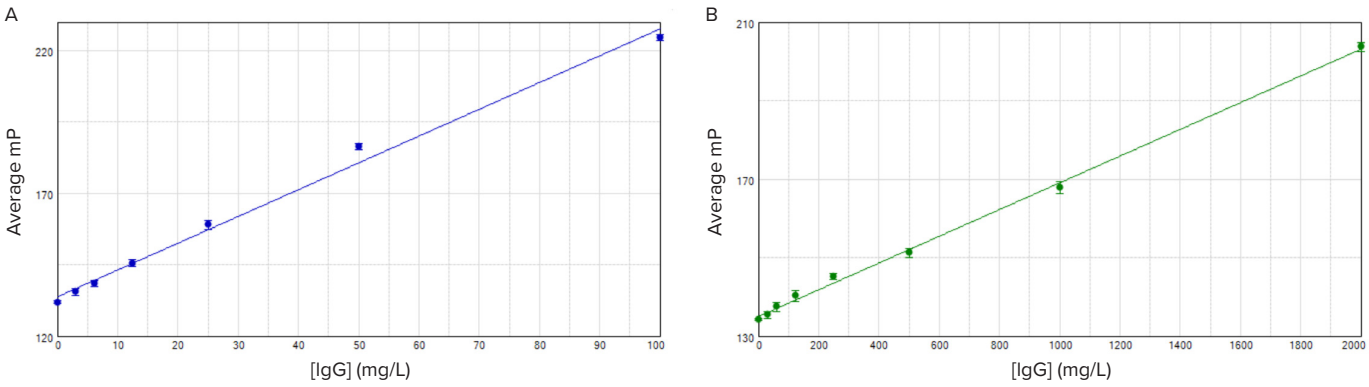


***Figure 4.*** *Standard curves for the ValitaTiter (A, $r^2$ = 0.993) and ValitaTiter Plus (B, $r^2$ = 0.998) assays. Curves were plotted using a linear curve fit in SoftMax Pro Software.*

| | iD5 | i3x | M5 | Paradigm |
|---|---|---|---|---|
| Delta mP | 93 | 70 | 80 | 89 |
| Average StDev (mP) | 1.12 | 2.12 | 0.57 | 0.82 |
| Average %CV | 0.7 | 0.7 | 0.5 | 0.3 |
| Max StDev | 1.54 | 3.53 | 1.06 | 1.27 |
| Max %CV | 1.0 | 1.3 | 1.0 | 0.4 |

***Table 4.*** *Standard delta mP, average standard deviation (StDev) and %CV (n = 4) for ValitaTiter standards read on Molecular Devices readers.*

| | iD5 | i3x | M5 | Paradigm |
|---|---|---|---|---|
| Delta mP | 69 | 85 | 92 | 88 |
| Average StDev (mP) | 1.05 | 3.54 | 1.63 | 1.14 |
| Average %CV | 0.7 | 1.3 | 1.6 | 0.4 |
| Max StDev | 1.59 | 6.49 | 2.26 | 1.43 |
| Max %CV | 1.1 | 2.0 | 2.4 | 0.5 |

***Table 5.*** *Standard delta mP, average standard deviation (StDev) and %CV (n = 4) for ValitaTiter Plus standards read on Molecular Devices readers.*

# Conclusion

The accurate and reliable measurement of mAb IgG titer is essential in the development and subsequent manufacture to ensure optimal cell culture performance for the production of all biologics. An assay that enables accurate results with a minimal investment of time and resources is critical to success. Here, we successfully demonstrate that ValitaTiter assays combined with Molecular Devices microplate readers enable quantitation of IgG across a wide functional range.

The ValitaTiter assay is a homogeneous, high-throughput method for precise and rapid quantitation of IgG in crude samples, without the requirement of sample preparation or purification steps. This 96-well assay has been fully validated on the SpectraMax iD5 reader and other Molecular Devices microplate readers with FP detection to ensure reliable results. SoftMax Pro Software minimizes setup time for detection and automates standard curve fitting and sample quantitation.

# Direct and rapid multi-attribute monitoring of intact monoclonal antibodies by icIEF-UV/MS

***Featuring an icIEF-UV/MS workflow using the Intabio ZT system from SCIEX***

Scott Mack, Zhichang Yang, Rashmi Madda, Jingwen Ding, Zoe Zhang, Rita Nichiporuk, and Maggie Ostrowski
SCIEX, USA

This technical note demonstrates a novel integrated workflow using the Intabio ZT system. This innovative system offers direct chip-based integration of imaged capillary isoelectric focusing (icIEF) with mass spectrometry (MS), which affords confident characterization of proteins and reliable quantitation of charge variants by icIEF-UV/MS. Further, the streamlined icIEF-UV/MS platform significantly reduces the time to results from weeks or longer to approximately 1 hour for routine samples, compared to ion exchange chromatography (IEX) with fraction collection.

Recombinant monoclonal antibodies (mAbs) have become an important class of biotherapeutics to treat a wide variety of diseases due to their high specificity, efficacy and flexibility.[1] During the manufacturing process, heterogeneity of the mAbs could occur due to enzymatic cleavage and chemical post-translational modifications (PTM).[2] Many of these PTMs, including deamidation, C-terminal lysine truncation, glycation and sialylation, change mAb charge (and, thus, isoelectric point or pI).[3] Characterizing the charge heterogeneity of mAbs is essential for critical quality attribute (CQA) assessment to ensure drug safety, efficacy and potency.[4] In addition, process changes can happen during manufacturing, which poses a significant concern for biopharma therapeutic development. Process changes can result in PTMs that impact product quality.

cIEF offers a high-resolution separation of protein charge variants, including compounds with similar pI values.[3] Therefore, cIEF is commonly used to monitor CQAs and product quality attributes (PQA). However, how to directly and rapidly identify and characterize different PTMs from these charge variant peaks in a single assay using iciEF-UV/MS data has been an unsolved problem for decades.

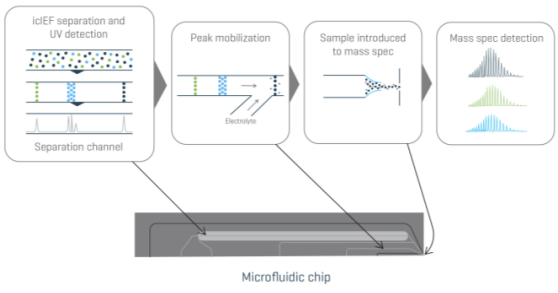


**Figure 1. icIEF-UV/MS analysis on the Intabio ZT system.** The Intabio ZT system directly integrates icIEF separation with a detection on the ZenoTOF 7600 system.

The Intabio ZT system aids in solving this challenge. It directly integrates icIEF separation with a ZenoTOF 7600 system, enabling direct coupling of icIEF charge variant analysis, UV quantitation and high-resolution mass spectrometry to identify intact proteins.

The UV results provide relative quantitation of the charge variant peaks. After focusing, separated charge variants are chemically mobilized and introduced to the MS through an on-chip electrospray ionization emitter for analysis without compromising separation efficiency and resolution. The icIEF-UV profile is correlated to the MS base peak electropherogram (BPE) for peak assignment and annotation.

The Intabio ZT system in combination with ZenoTOF 7600 system confidently identified numerous attributes, including deamidation and low-abundance glycation events. The total turnaround time for routine samples is approximately 1 hour, which offers significant time savings compared to the conventional LC IEX workflow and fraction collection followed by MS identification. Since all data are collected in a single MS and optical analysis, data integrity is superior to fraction collection for GLP considerations.

## Key features

- Seamless identification of charge variants with a microfluidic chip-based integrated icIEF-UV/MS technology. The Intabio ZT system is exclusively coupled to the ZenoTOF 7600 system
- Platform offers both rapid multi-attribute monitoring of intact biotherapeutics and comprehensive characterization by icIEF-UV/MS
- The 30-min sample analysis is significantly faster than conventional cIEF and IEX workflows requiring fractionation for the following identification.
- icIEF separation and UV quantitation correlate well with standard icIEF techniques

- Streamlined, intuitive data analysis software for rapid reporting and result sharing

## Methods

***Equipment:*** Intabio ZT system (SCIEX) and Intabio ZT cartridge (SCIEX, P/N 5088248) were used for the separation of NISTmAb and its charge variants. MS detection was performed on the ZenoTOF 7600 system (SCIEX, P/N 5080337) equipped with OptiFlow interface components (SCIEX, P/N 5084645).

***Chemicals and reagents:*** The Intabio system – Electrolyte and Mobilizer kit (P/N 5088205) was used for anolyte, catholyte and mobilizer. Anolyte and mobilizer were used undiluted. The stock catholyte solution was 1% and diluted to 0.25% for use in the reagent drawer. The stock anolyte is 1% formic acid and catholyte is 1% diethylamine. The mobilizer is composed of 25% acetic acid, 25% acetonitrile and 50% water.

A 500mM cathodic spacer solution containing free base L-arginine (Arg) (purity ≥ 98.5%, Sigma-Aldrich, P/N A8094-25G) was prepared by dissolving 0.870 mg of Arg powder into 10 mL of Milli-Q water. The electrolytes and cathodic spacer solutions were stored at room temperature. pI markers (CanPeptide) were individually dissolved in Milli-Q water at 5 mg/mL.

Prior to icIEF-UV/MS analysis, NISTmAb was desalted with a Zeba Spin Desalting Columns, 7K MWCO, 0.5 mL(Thermo Fisher Scientific, P/N 89882).

***icIEF-UV/MS analysis:*** MS signal was optimized before sample analysis using a solution containing 100 µg/mL NISTmAb in mobilizer solution. The NISTmAb solution was infused through the Intabio ZT cartridge with 100-150 mbar of pressure, and electrospray was achieved into a ZenoTOF 7600 system with a tip voltage between 5000 and 5500 Volts. Nitrogen gas was applied between 60 to 75 psi to the on-chip integrated nebulization channels coplanar with the tip of the icIEF-UV/MS chip to achieve stable and robust ionization and sample entry to the MS.

**Table 1. icIEF separation**

| Hold time [s] | Anode voltage[V] | Cathode setting | Mobilization setting | Step |
|---|---|---|---|---|
| *60* | *1500* | *0V* | *0A* | *Focusing* |
| *60* | *3000* | *0V* | *0A* | *Focusing* |
| *300* | *4500* | *0V* | *0A* | *Focusing* |
| *600* | *8500* | *0A* | *5500V* | *Mobilization* |

Samples containing 250 µg/mL NISTmAb, 10 mM arginine, 1% Pharmalyte 3 to 10 (Cytiva, PN 17045601), 2.5% Pharmalyte 8 to 10.5 (Cytiva, PN 17045501), and 6.25 µg/mL peptide pI markers were vortexed and then degassed by centrifugation at 3900 rcf.

The streamlined process of icIEF-UV/MS analysis is illustrated in Figure 1. First, 70 psi of nitrogen was supplied to the internal nebulization channel within the Intabio ZT cartridge during the loading, focusing, and mobilization cycles of the icIEF-UV/MS separation process. After priming the channels within the Intabio ZT cartridge with either electrolyte solution or water, 50 µL of the sample was automatically loaded onto the separation channel using the autosampler. The icIEF separation was achieved using the parameters shown in Table 1. Absorbance measurements were collected at 1 Hz throughout the focusing and mobilization steps. The samples were introduced into the ZenoTOF 7600 system by a metered 2 µL/min flow of chemical mobilizer, and the data was acquired using parameters shown in Table 2.

**Table 2. TOF MS parameters.**

| Parameter | Value |
|---|---|
| *Curtain gas* | *15 psi* |
| *Spray voltage* | *5500 V* |
| *TOF start mass* | *2000 m/z* |
| *TOF stop mass* | *6000 m/z* |
| *Accumulation time* | *0.5 s* |
| *Source temperature* | *100ºC* |
| *Declustering potential* | *210 V* |
| *Collision energy* | *55 V* |
| *Time bins to sum* | *150* |

**Data processing:** UV profiles and mass spectra from the icIEF-UV/MS analysis of NISTmAb were analyzed using the Biologics Explorer software. Each peak in the icIEF-UV profile was integrated to determine peak area and percent composition. Intact masses were estimated from the raw mass spectrum under each peak of the icIEF-MS profile utilizing a charge deconvolution algorithm with a mass range setting between 145,000 and 150,000 Da.

## Results and discussion

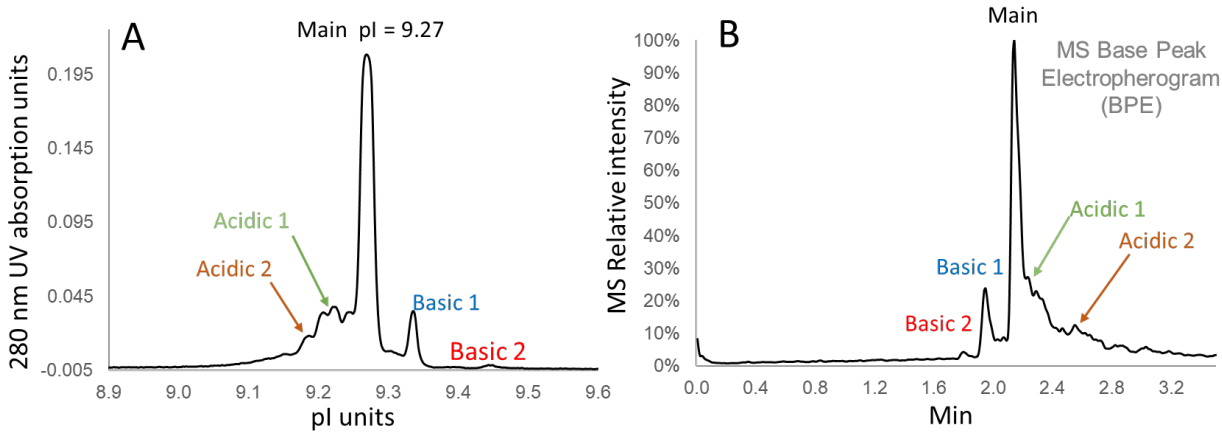


**Figure 2. NISTmAb intact charge profiles.** Panel A shows an icIEF-UV profile of charge variant separation, in the scale of the pI value (x axis) acquired from the Intabio ZT system. Panel B shows an MS base peak electropherogram (BPE) of mAb charge variants acquired from ZenoTOF 7600 system after icIEF separation.

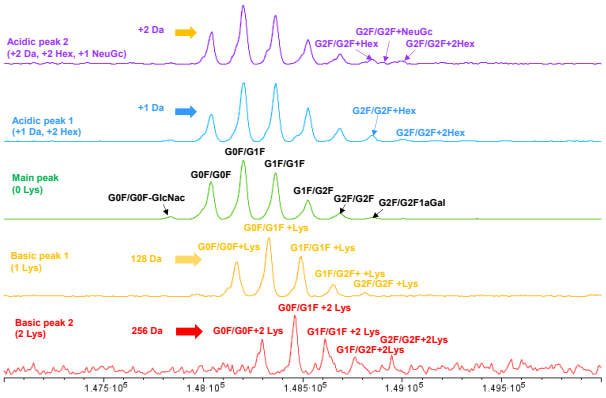


**Figure 3.** Overlay of the intensity normalized deconvoluted intact mass spectrum of the 5 NISTmAb charge variants, showing the identity of the peaks detected in the icIEF-UV/MS analysis (Figure 2); the data analysis was performed by the Biologics Explorer software.

Figure 2 shows a separation profile of the charge variants of NISTmAb acquired with the Intabio ZT system coupled to ZenoTOF 7600 system. Panel A shows a 280-nm UV absorbance profile of NISTmAb charge variants separated by icIEF. Panel B is a MS BPE of the corresponding peaks after mobilization, electrospray and detection by MS. The separation profiles generated by icIEF-UV (once inverted) and icIEF-MS on the Intabio ZT system and ZenoTOF 7600 system, respectively, are similar. icIEF-UV profile shows separated peaks across the icIEF separation channel, whereas the MS data is acquired based on the time the charge variants are mobilized and electrosprayed into the mass spectrometer. Basic peaks of the icIEF-UV profile with high pI are introduced first into the MS system for analysis and therefore appear at earlier time points (left side of the MS BPE). The MS BPE shows that all the peaks observed from icIEF-UV profile were also detected by the MS system without compromising separation resolution. This result indicates that the separation efficiency of charge variants was well maintained after chemical mobilization. The entire focusing and separation only took 15 min, demonstrating the capability of high-throughput characterization of charge variants.

A detailed analysis of the charge variant peaks was performed on intact NISTmAb. Figure 3 shows the intensity normalized deconvoluted mass spectra of 5 charge variants from the NISTmAb sample. The basic peaks 2 and 1 charge variants show +256 and +128 Da mass increases compared to the main peak, which are respectively identified as C-terminal lysine variants.

Additional peaks above G2F/G2F at +162 Da intervals are observed in acidic peaks 1 and 2, identified as putative glycation. Masses that correspond to glycans with NeuGc sialic acid are observed in acidic peaks 2. A +1 Da mass shift was observed in acidic peak 1 and a +2 Da mass shift was observed in acidic peak 2 for proteoforms with G0F/G0F, indicating a high abundance of proteoforms with1 deamidation or 2 deamidation events in these 2 charge variants. Due to the excellent charge heterogeneity separation on the Intabio ZT system, neutral proteoforms, such as the glycosylation series, including low-abundance glycans such as G0F/G0F-GlcNAc, are readily measured by icIEF-UV/MS.  In a follow-up technical note, we

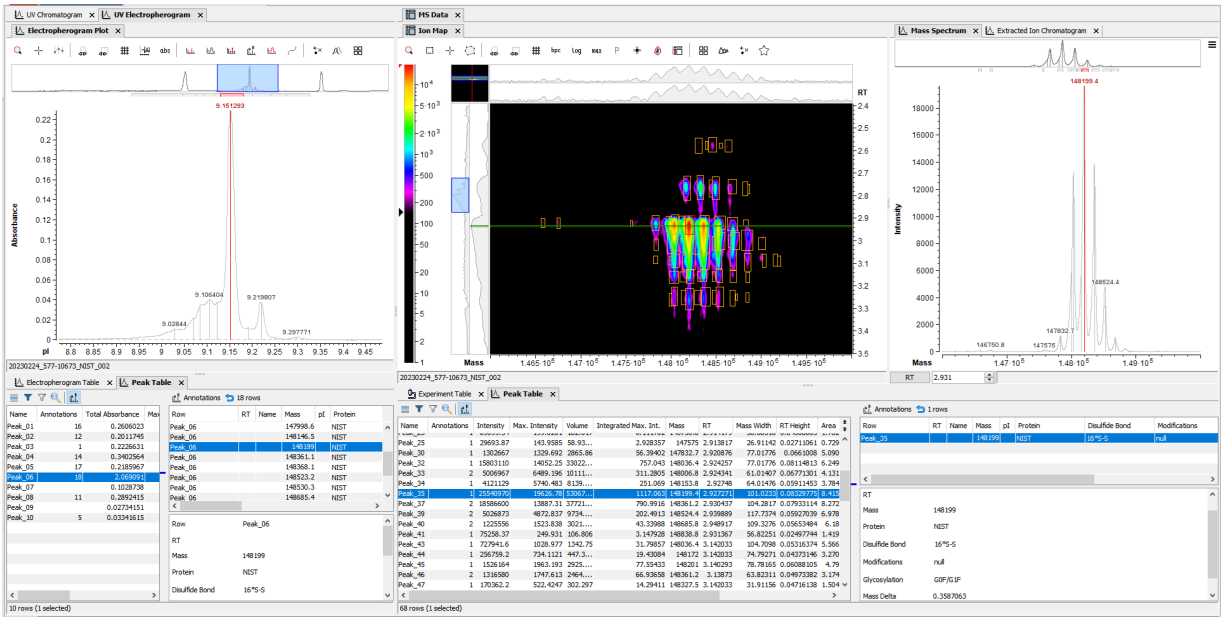

**Figure 4. Results interface in the Biologics Explorer software.**

confirm the existence of glycation by performing the icIEF-UV/MS analysis on a deglycosylated NISTmAb using the Intabio ZT system.

Figure 4 shows an example of the analysis result using the Biologics Explorer software. The layout simultaneously includes the UV electropherogram, results summary, MS data, deconvoluted ion map results and raw MS data. On the top left panel, the UV electropherogram illustrating the isoelectric focusing separation of charge variants and the integration of identified peaks. Peaks identified in the icIEF-UV profiles (electropherogram table) and MS BPE (peak table) are separately listed in the bottom left panel with detailed annotations of the identified proteoforms in the annotations section. The top right panel presents the ion map (color image) and deconvoluted mass spectra of the peak selected in the left panel. The bottom right panel shows the peaks selected in the deconvoluted mass spectra, including peak intensities, retention time and method used for data analysis. This report summary demonstrates a comprehensive overview of a streamlined charge variant characterization using the Biologics Explorer software.

## Conclusions

- The Intabio ZT system demonstrates a streamlined workflow to separate charge variants by icIEF and identify them with ZenoTOF 7600 system
- A unified icIEF-UV/MS workflow offers high sensitivity to detect low-abundant charge variants that might impact product quality
- The separation efficiency and resolution of icIEF are well-maintained after mobilization, enabling highly confident identification of CQAs by icIEF-UV/MS, such as C-terminal lysine variants, deamidation and glycation (Hex)
- The Intabio ZT system is a commercially available platform that offers workflow combining icIEF separation, UV quantitiation and MS-based identification.

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Enabled by

# Automation

Increase workflow reproducibility and standardization with cutting edge tools

APPLICATION NOTE

# Consistent Cell Maintenance and Plating through Automation


## Summary

- Used the Biomek i7 Workstation to maintain adherent and suspension cell cultures at high viability
  - Integrated Tilting ALP enabled low-density plates to be used for culture
  - Integrated Vi-CELL XR Cell Viability Analyzer assessed cell viability
  - "Split Volume" option allowed viable cells/mL value to drive passage volume with no scripting
- Automated concomitant cell plating into 384-well plates
  - 1200 µL multichannel head enabled mixing of large volume reservoirs for even cell dispersion
  - Efficient multidispense function gave consistent plating across wells


Cell culture is a valuable yet time-consuming aspect of basic biological research and drug discovery. Whether short- or long-term assays, the maintenance of cells is a frequently recurring process and the workload and possibility for contamination increases as the number of cell lines being used by a laboratory increases. In addition, different scientists frequently use different techniques when maintaining cell lines and these alterations can manifest in subsequent assay variability when using these cells.

Here we demonstrate the automation of all aspects of standard cell culture and plating on a Biomek i7 Automated Workstation (Figure 1). A sterile working environment was maintained with HEPA-filtered fans on the Biomek i7 enclosure and the use of sterile Biomek tips prevented contamination throughout the weeks-long culture process. The ability of the Biomek i-Series instruments to directly access integrated devices such as incubators, plate and tip storage, and various analyzers makes these systems ideal for cell culture and cell-based assays.

**Figure 1.** Biomek i7 with HEPA filters and integrated devices.

CHO cells were cultured under adherent conditions in 6-well plates and a Tilting ALP was used to angle the plate to enable the Span-8 probes to completely remove culture media without disturbing the cells. Cells were then trypsinized by incubating on a heated Peltier device, resuspended and counted on an integrated Vi-CELL XR Cell Viability Analyzer. 1 X $10^6$ viable cells were passaged into the next well every three days utilizing the "Split Volumes" option to automatically account for when transfer volumes were greater than the tip capacity. 24 hours after plating, cell confluence was determined on four regions of each well using a SpectraMax i3X Multi-Mode Detection Platform with SpectraMax MiniMax 300 Imaging Cytometer. Figure 2 shows the distribution of cells after passaging manually or on the Biomek i7 Workstation. The lack of cell clumps in the images shows the automated trypsinization was effective and the low CV value illustrates even distribution of cells across the well.

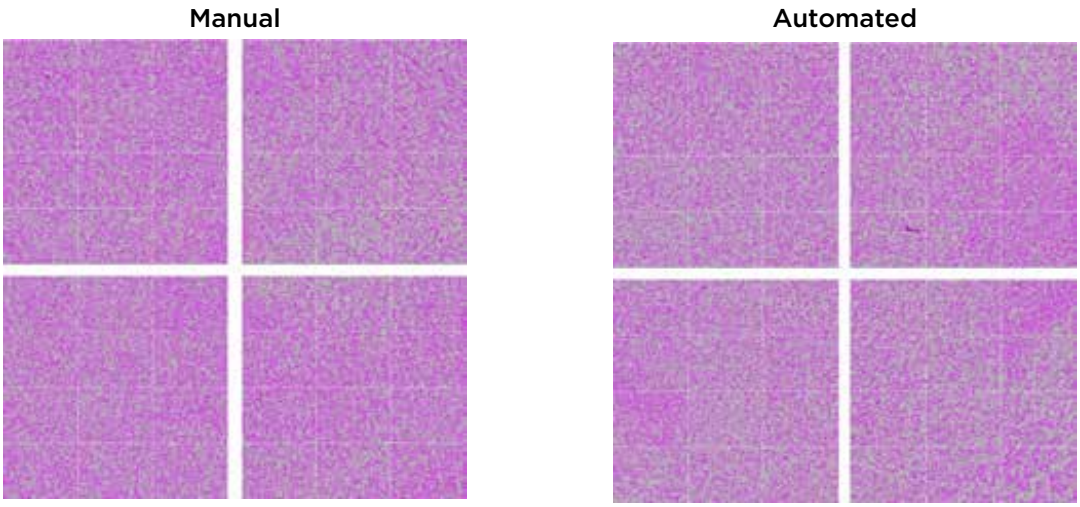
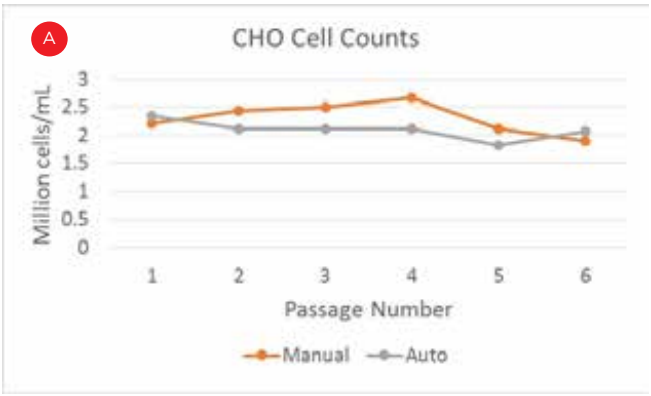
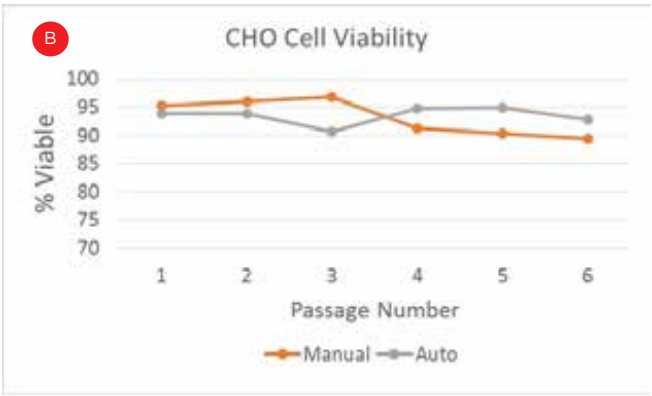



**Figure 2.** Consistent automated cell passage. Cell confluence (purple) was measured across 12 images in each of 4 regions of the 6-well plate well 24 hours after manual and automated passaging of 1 X $10^6$ cells. Average confluence and CVs across the 4 regions illustrate even cell distribution and comparable results between automated and manual passages.

Figure 3 plots cell counts and viability of CHO cells over six automated and manual passages, as measured on the Vi-CELL. The Biomek i7 Workstation was able to maintain the cells to comparable levels as the manual passaging results while maintaining cells above 90% viability. The average cell count across passages was slightly lower for automated passage, likely due to the inability to access a small portion of the well when tilted. Most significantly, the variability across passages is lower for the automated system than what was achieved manually (CV = 7.9% vs. 13.8% for cell counts), and this consistent treatment should aid assay consistency.



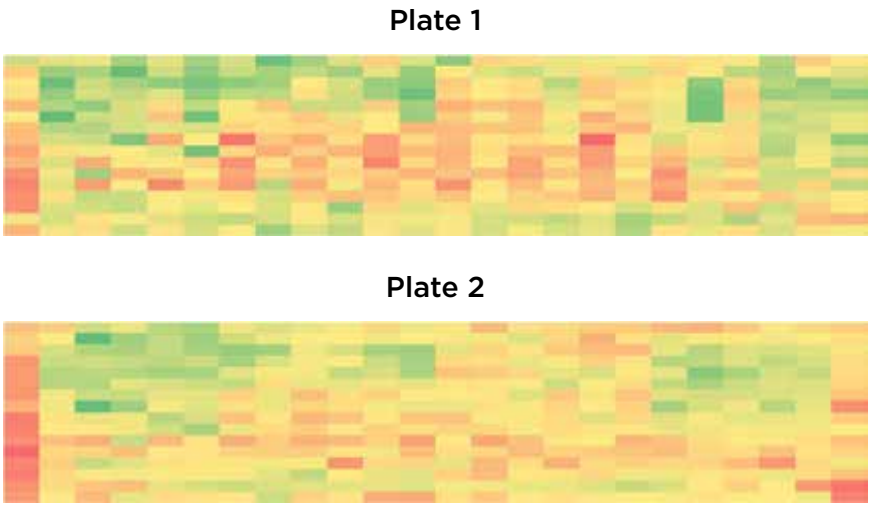

C

| | Avg. Count | %CV | Avg. Viability | %CV |
|---|---|---|---|---|
| **Manual** | 2.38 X $10^6$ | 13.8% | 93.9% | 3.7% |
| **Automated** | 2.12 X $10^6$ | 7.9% | 93.9% | 1.9% |

**Figure 3.** Automated cell maintenance. CHO cell counts (A) and viability (B) following 6 manual and automated passages, with comparable results between the two approaches. C) Averages and CVs across the six passages. Automated passaging resulted in lower variability in both metrics.

To demonstrate cellular assay plating, 1 X $10^6$ cells were added to 100 mL media in a large reservoir during the culture process. The 1200 µL capacity Multichannel-96 head was used to mix the media to ensure even cell distribution throughout the reservoir. 1000 cells were then multidispensed into all quadrants of two 384-well plates in a single transfer step. 24 hours after plating, cell counts were measured in each well on the SpectraMax MiniMax. Figure 4A shows the heat maps of cell counts for each plate, showing that no bias was seen towards any portion of the plates. This was confirmed by CVs below 10% when comparing average cell counts across columns or rows for each plate (Figure 4B). In addition, the consistent plating from the multidispense step is demonstrated by CVs below 10% when comparing the average cell count for each of the 8 quadrants. This mix and multidispense was executed in under 2 minutes, thereby enabling high-throughput applications when compared to the repeated mixing and roughly 96 transfers it would take to execute this plating manually.

A




B

| | Column CV (24) | Row CV (16) | Quadrant CV (8) |
|---|---|---|---|
| **Plate 1** | 8.1% | 7.6% | |
| **Plate 2** | 8.2% | 6.8% | |
| **Combined** | | | 7.0% |

**Figure 4.** Automated cell plating. A) Heat maps of cell counts in each well of two 384-well plates showing even distribution across the plates. B) CVs of average cell counts across columns and rows for each plate and for the average across the 8 quadrants between the two plates illustrate thorough mixing of cells in the reservoir and consistent multidispensing respectively.

Automation of this simple yet frequently burdensome process of passaging and plating cells demonstrates the power and flexibility of the Biomek i7 Workstation. The Span-8 pod enabled processing of low-density plates (i.e. 3 mL culture in 6-well plates) while the large capacity multichannel head enabled rapid plating of 768 assay wells. Integrated cellular analyzers gave the ability to utilize cell count data to drive the cell passaging process and image the passaged and plated cells without intervention, thereby reducing the likelihood of human error.

The suite of SAMI software can manage the processing of multiple plates through the integrated system and track where cells are within a multi-day or multi-week application. In addition, DART software acts as a data repository that stores data throughout a process or between processes and ensures that the data is easily accessible for additional analysis. By utilizing the Biomek i-Series instruments to automate workflows that are used frequently, the bench time saved by scientists accumulates quickly and these efforts can be redirected towards more valuable pursuits such as experimental design and data interpretation.

APPLICATION NOTE

# Automation of IgG Quantification using the Biomek i7 Hybrid Automated Workstation

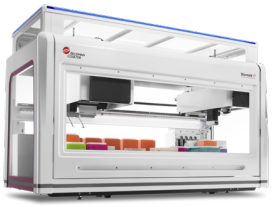
*Beckman Coulter Life Sciences, Indianapolis, IN*

## Introduction

Over the last 30 years, biotherapeutics, namely monoclonal antibodies (mAbs), have become an important class of drug molecules. The first mAb to achieve FDA approval was Orthoclone OKT3 (muromonab-CD3), which was approved in 1986 for the treatment of tissue rejection in transplant recipients.[1] Since then 100 mAbs have received FDA approval (or emergency use authorization), and each year an increasing number of biotherapeutic drug candidates are tested in clinical trials.[2] As their name implies, biotherapeutics are drug molecules that are derived from a biological source.[3] Due to advances in bioprocessing and cell line engineering, Chinese hamster ovary (CHO) cells have become one of the leading platforms for mAb production, accounting for a large majority of the clinically relevant biotherapeutics.[3,4] An important factor in the production of these drug candidates is the selection of a single clone (i.e. monoclonal) that is ideal for mAb production and scale-up. Clones are generally adapted to suspension culture in serum-free (ADCF) and evaluated for their growth rates and ability to reach high cell densities. Increased biomass can correlate with increased antibody production (productivity), which is another critically important metric for an ideal clone.[5]

Evaluation of clone productivity is one of the early steps in the cell line development process for CHO-based biotherapeutic drug discovery. Clones are tested for their ability to generate the protein of interest, and low-producing clones are discarded in favor of high producers. Most commercially available mAbs are of the immunoglobulin G (IgG) isotype, so the relative volumetric productivity (or titer) of clones can be approximated by measuring the amount of IgG secreted into the cell culture medium.[5] As quantification of IgG is critical to selecting the right clone(s), a number of methodologies have emerged to measure IgG levels in culture medium. Traditionally, enzyme-linked immunosorbent assays (ELISA) and high-performance liquid chromatography (HPLC) have been used for IgG quantification, but these methods suffer from being time-intensive, and the sample preparation process is laborious. Biolayer interferometry is another popular method. It is preferable to the methods mentioned above as it is high throughput and amenable to automation,[6] but it requires the use of specialized equipment. As mAb discovery efforts often involve screening 1000+ clones,[4] there is a need for faster, plate reader-based methods. Further, if these assays were amenable to laboratory automation, results could be obtained faster with less work required by the user. This would reduce the time and resources consumed in the early stages of the clone selection process.

The first assay selected was the Easy-Titer kit, which is an absorbance-based method to measure IgG concentrations. In this assay, the presence of IgG causes the clumping of specially sensitized, absorbent microspheres, and this clumping results in an IgG concentration-dependent decrease in absorption.[7] This is a simple assay that requires fast, accurate pipetting, making it an ideal candidate for laboratory automation (Figure 1). Another relatively new assay that is gaining attention is ValitaTiter. This a fluorescence polarization-based method that uses a fluorescently labeled Ig binding protein so that increasing concentrations of IgG lead to increased polarization (Figure 1).[8] Notably, both methods can be performed directly on cell culture medium; no pre-assay IgG purification is required prior to IgG quantification. Here, we sought to automate these two plate-based IgG quantification assays using a Biomek i7 Hybrid automated workstation (Figure 2).



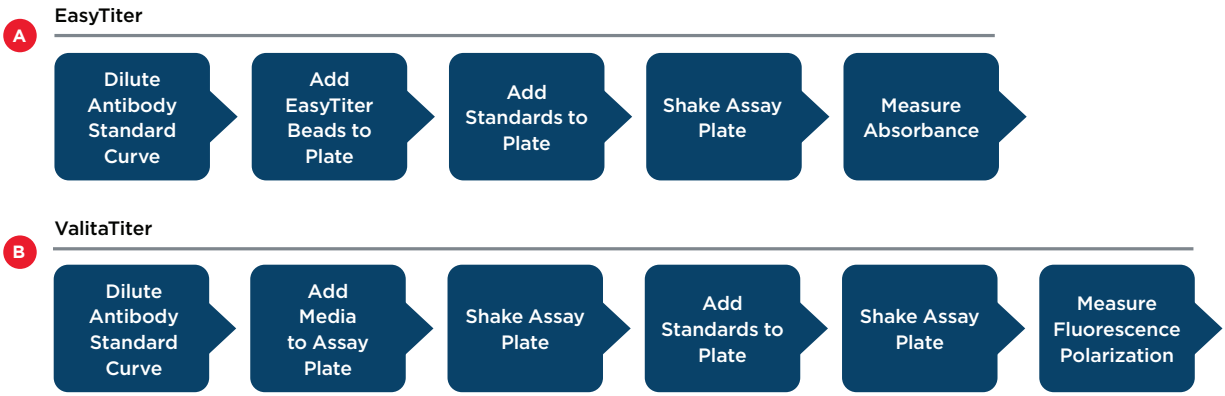



**Figure 1. IgG Quantification Workflows.** Flowcharts of ThermoFisher Easy-Titer **(A)** and ValitaCell ValitaTiter **(B)** IgG quantification assays. Steps performed using the automated Biomek method are highlighted in blue.

The Biomek i7 Hybrid Automated Workstation is an automated liquid handler that is capable of efficiently performing the complex liquid handling steps required to quantify IgG samples (Figure 2). This minimizes the number of required user interactions and increases walkaway time, freeing the operator to attend to other laboratory tasks. The multichannel pod can be equipped with a 96-well head that can accurately pipette 1 to 1200 µL or a 384-well head that is accurate over the range of 0.5 to 60 µL. Additionally, the 8-channel Span-8 pod is accurate from 1 to 1000 µL. The Biomek i7 hybrid automated workstation supports 45 deck positions and can be directly fitted with orbital shakers, heating/cooling Peltiers, and tip-washers for plate and sample processing (Figure 2). Further, depending on user needs, the Biomek i7 hybrid automated workstation supports integration with other automated plate handling instruments, such as thermal cyclers, incubators, barcode readers, plate washers, multimode plate readers, centrifuges, and more. Here, we demonstrate automated IgG quantification using the Easy-Titer and ValitaTiter assays on a Biomek i7 workstation. The automated assay workflows provide excellent results that are equivalent to manually processed samples, and automation can reduce the hands-on time and possibility of sample handling errors by the user.


**Figure 2.** Biomek i7 Hybrid Automated Workstation.

## Methods

### Easy-Titer Assay

The Easy-Titer assay was performed according to manufacturer instructions, and all liquid handling steps were executed both manually and using an automated method on a Biomek i7 hybrid workstation. A standard curve ranging from 500 ng/mL to 8 ng/mL of human IgG isotype control antibody (Invitrogen) was generated in the assay kit's Dilution Buffer. Next, 20 µL of Easy-Titer beads were added to each well of a 96-well clear, flat bottom plate (Beckman Coulter Life Sciences). Then, 20 µL of IgG standard was added to the assay plate and the plate was shaken for 5 minutes using an on-deck Orbital Shaking ALP. Then, 100 µL of Blocking Buffer was added to each well and the plate was again shaken for 5 minutes. Finally, the plate was transferred to an integrated SpectraMax i3x (Molecular Devices) and absorbance was measured at 405 and 340 nm.

### ValitaTiter Assay

Like above, the ValitaCell ValitaTiter assay was performed according to manufacturer instructions, and all liquid handling steps were executed both manually and using an automated method on a Biomek i7 hybrid workstation. First, 60 µL of assay buffer (OptiMEM) was added to each well of the black, 96-well, half-area ValitaTiter plate, and the plate was shaken for 5 minutes using the on-deck Orbital Shaking ALP. Then, the standard curve was generated by serially diluting a human IgG antibody from 100 mg/L to 3 mg/L in OptiMEM buffer. Next, 60 µL of the standard curve was added to the ValitaTiter assay plate, and the plate was shaken for 5 minutes. Finally, the plate was transferred to an integrated SpectraMax i3x (Molecular Devices) equipped with a fluorescence polarization detection cartridge, the plate was incubated in the dark for 3 minutes, and polarization was measured.

### Data Analysis

All data was analyzed using Prism 9 software (GraphPad). For Easy-Titer experiments, absorbance was plotted as a function of the log of the concentration of IgG antibody, and the data were fit to a line. For ValitaTiter, polarization (in millipolarization units, mP) was calculated using the following equation, where $F_{Perp}$ and $F_{Para}$ are the fluorescence in the perpendicular and parallel channels, respectively.

$$\text{Polarization (mP)} = 1000 \times \frac{F_{Para} - F_{Perp}}{F_{Para} + F_{Perp}}$$

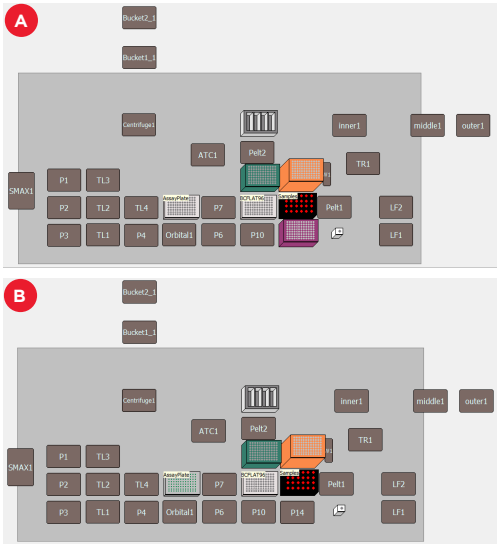



**Figure 3. Biomek i7 Hybrid Deck Layouts.** Deck layouts of automated ThermoFisher Easy-Titer **(A)** and ValitaCell ValitaTiter **(B)** IgG quantification assays. Together the automated method required the following deck components: 7 1X1 ALPs, Orbital Shaking ALP, and an integrated Molecular Devices SpectraMax i3x.

## Results and Discussion

In order to validate the performance of the newly developed, automated Easy-Titer method, results generated using the Biomek i7 workstation were compared to manual experiments (Figure 4). The Easy-Titer assay kit allows for absorbance measurements at two different wavelengths: 340 nm (Figure 4A) and 405 nm (Figure 4B). A control human IgG was tested at concentrations ranging from 500 ng/mL to 8 ng/mL in triplicate, and absorbance was measured. Both the automated and manual method results displayed excellent linearity over this IgG concentration range. When absorbance was measured at 340 nm, the $R^2$ values were 0.923 and 0.966 for the manual and automated methods, respectively (Figure 4A). The datasets generated at 405 nm exhibited $R^2$ values of 0.913 and 0.946 for the manual and automated methods, respectively (Figure 4B).

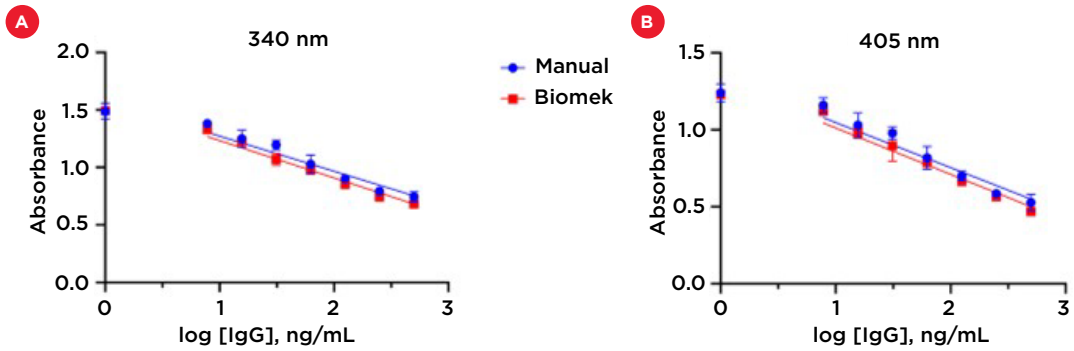



**Figure 4. Easy-Titer Human IgG Standard Curve.** Results of Biomek (Red) and manually (Blue) performed Easy-Titer assays. Absorbance was measured at 340 nm **(A)** and 405 nm **(B).** Data represent mean ± S.D. of n=3 replicate wells.

To confirm the accuracy of the automated ValitaTiter method, standard curves were generated using the Biomek i7 workstation and manual experiments (Figure 5). The control human IgG was tested at concentrations ranging from 100 mg/L to 3 mg/L in triplicate, and fluorescence polarization was measured. Both the automated and manual method results displayed excellent linearity over this IgG concentration range, with $R^2$ values of 0.9966 and 0.9889, respectively (Figure 5). This data, together with the Easy-Titer data above (Figure 4), show that the Biomek i7 workstation can perform various methodologies to quantify IgG over a wide range of concentrations.

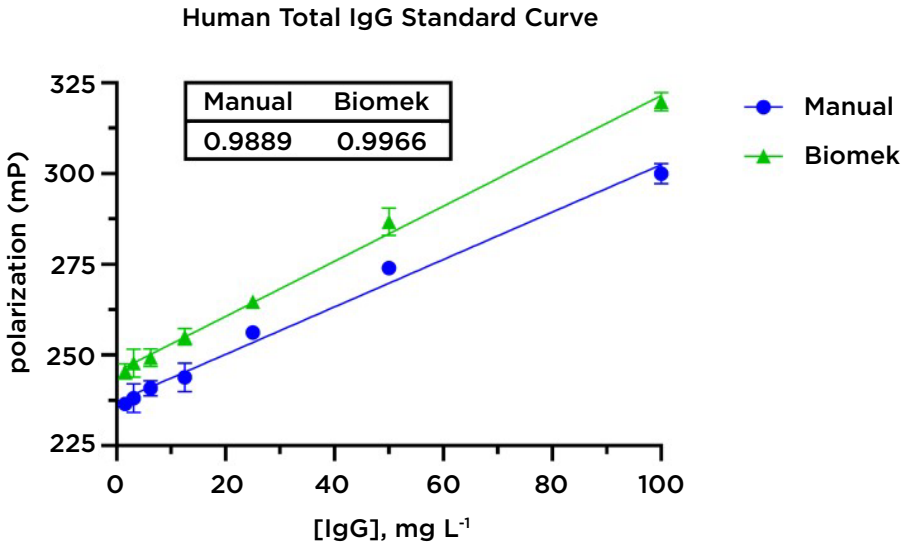



**Figure 5. ValitaTiter Human IgG Standard Curve.** Results of Biomek (Green) and manually (Blue) performed ValitaTiter fluorescence polarization assays. Data represent mean ± S.D. of n=3 replicate wells.

## Summary

Together the data presented here shows IgG quantification is amenable to automation using a Biomek i7 hybrid automated workstation, providing a user-friendly, hands-free method with increased throughput.

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## Materials

| Equipment | Manufacturer |
|---|---|
| Biomek i7 hybrid automated liquid handler<br>Orbital Shaking ALP | Beckman Coulter Life Sciences |
| SpectraMax i3x | Molecular Devices |

**Table 2.** Instruments.

| Equipment | Manufacturer | Part Number |
|---|---|---|
| Easy-Titer Human IgG (H+L) Assay Kit | Thermo Fisher | 23310 |
| Human IgG Isotype Control, 10 mg | Invitrogen | 31154 |
| OptiMEM | Gibco | 31985-070 |

**Table 3.** Reagents.

| Consumables | # | Manufacturer | Part Number |
|---|---|---|---|
| Biomek i-Series, 230 µL pipette tip | 1 | Beckman Coulter Life Sciences | B85903 |
| Biomek i-Series, 1070 µL pipette tip | 1 | | B85940 |
| Biomek i-Series, 90 µL pipette tip | 1 | | B85884 |
| Biomek 96-well microplate | 2 | | 609844 |
| ValitaTiter 96 IgG Plates | 1 | ValitaCell | VAL003 |

**Table 4.** Consumables used.

Enabled by

# Data

Enterprise level workflow management, data handling and analyses

# Customer success story: streamlined cell line development

This success story demonstrates how an IDBS customer was able to reduce the time to create cell line development reports by 50%. The IDBS platform supports hundreds of users globally at a multinational biotechnology company focusing on biologic therapeutics.

## The challenge

**Six to eight weeks to generate a cell line development report**

The cell line development group was struggling to generate reports. A hybrid paper, ELN and Excel-driven landscape hindered the creation of reports to release cell line cultures. IDBS worked with the Customer to understand the current challenges and bottlenecks. Given the hybrid paper/digital operating environment, it was estimated that more than 32,000 resource hours per year were spent manually connecting processes, data, materials, equipment and people, together. It took six to eight weeks to generate a final cell line development report.

## The solution

The Customer accepted IDBS' recommendation to conduct a series of workshops to map out their cell line development processes, business rules and desired flexibility. The graphic below is a generic output of the developed process map. Each line represents a flow of structured data and information that can be easily modified and extended in a GxP-compliant manner.

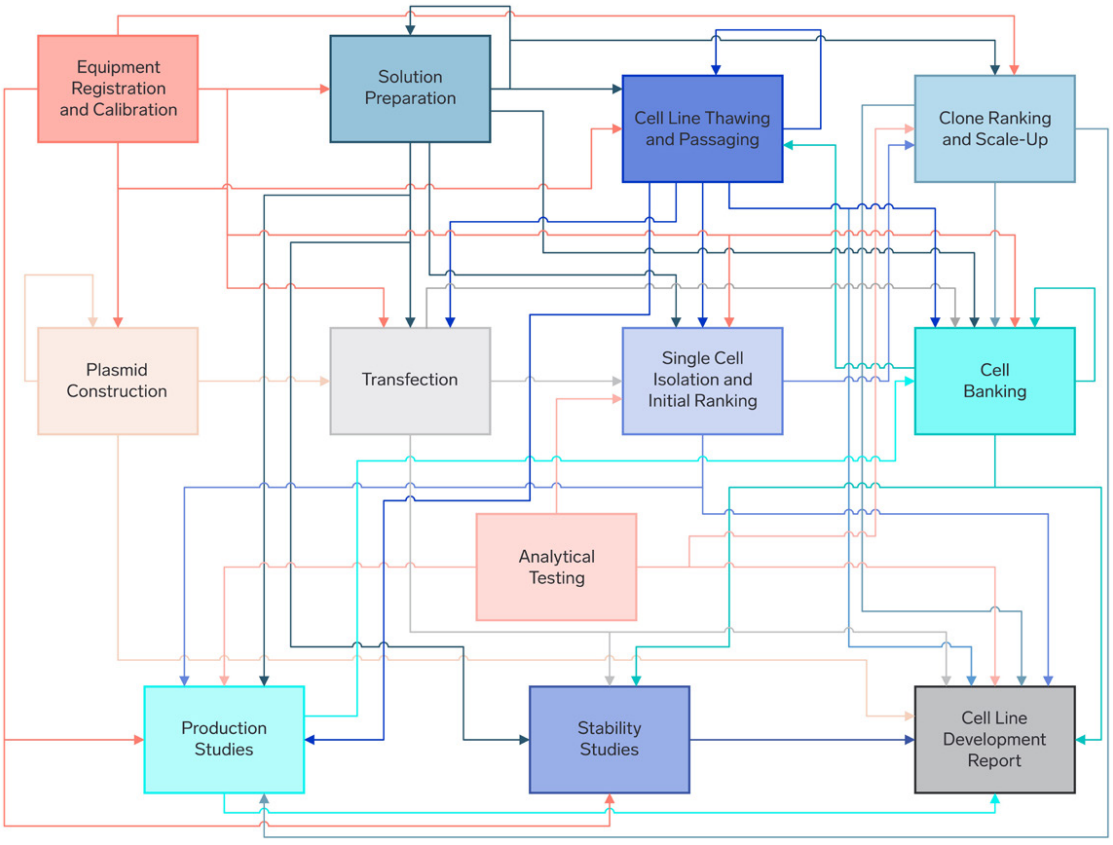


Fig. 1. Information and data flow throughout cell line development

## The results

IDBS worked with the Customer to estimate the current time spent on core activities and expected time savings. After implementing IDBS' digital workflows for structured data capture, the Customer calculated actual time savings of over 14,000 resource hours per year (Fig. 2). The time to generate a final cell line development report was calculated to be two to three weeks, which is a 50% reduction.

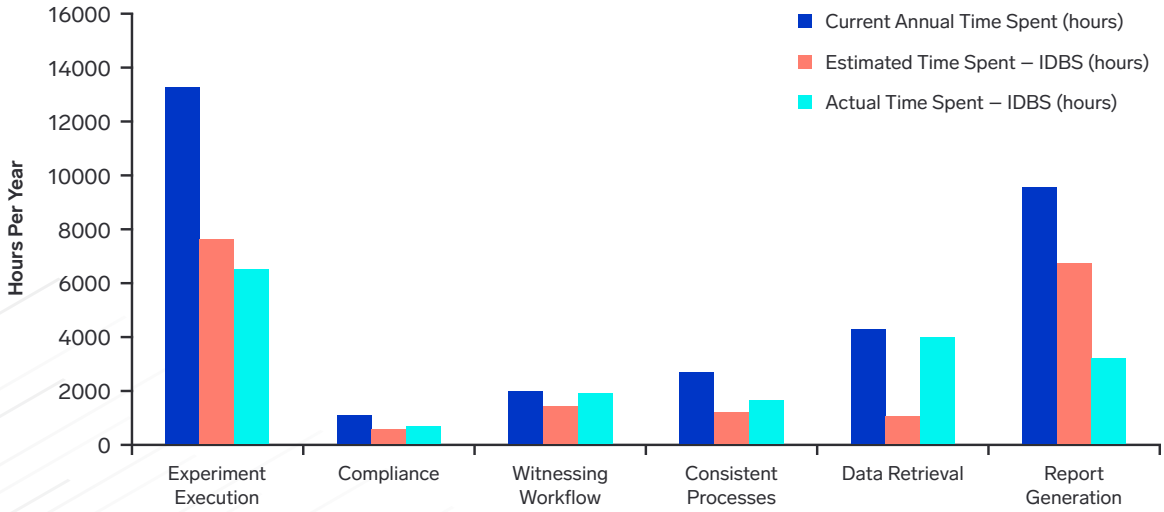


Fig. 2. Estimated vs. actual time savings by activity

## Additionally, the Customer's own success team reported:

**50% reduction in time to generate a cell line development report**

- The ability to generate technical reports against searchable data enabling scientists to search for cell culture data directly
- Reduced error resolution times because alerts were added to flag data errors and inconsistencies in the clone selection process, which called immediate attention to issues
- As a result of the increased efficiency, there was a 25% increase in lab capacity to support additional projects.

Business rules to elevate a target to the next step can be applied or adjusted within each workflow to expand or contract the "hits" based upon specific acceptance criteria. The dataflow is preserved, and managers are presented with consistent, accurate and complete data associated with the generation of a master cell bank including product quality, clonality and plasmid construction.

## IDBS' approach to bioprocess development digital workflows is:

- **Flexible** – Our platform accommodates both flexible non-GxP and controlled GxP workflows
- **Standardized** – Digital workflows ensure consistency and comparability
- **Structured** – Process data capture, storage and retrieval
- **Traceable** – Genealogy and material consumption is recorded across all process steps
- **Compliant** – Meets the FDA's 21 CFR Part 11 guidelines for GxP operations

## The IDBS difference for biologics development

Biologic therapeutics process development is a complex, iterative and multi-disciplinary activity. Before electronic lab notebooks (ELNs) became widely available, this work was typically recorded in paper lab notebooks, which meant senior scientists had to spend hours printing, cutting and pasting Excel and instrument printouts. While ELNs eliminate paper, electronic "paper on glass" systems don't solve the more fundamental challenge of enabling scientists to easily find, compare and reuse information. Complex development reports spanning months of activity still take weeks of effort to prepare and data integrity issues uncovered at the eleventh hour can delay regulatory submissions by as much as six months.

Since 2008, IDBS has been implementing both large-scale and modular deployments of bioprocess development solutions designed to provide structured data capture through digital workflows with integrated instrument connectors. While biologics process development is challenging, IDBS believes that managing and integrating your workflows and data shouldn't be.

Genedata
solutions in silico

MERCK

> ***Genedata Expressionist provides high-quality qualitative and quantitative results and its flexible workflow-based approach makes it the ideal platform for processing and analyzing mass spectrometry data generated using our innovative methods.***
>
> Lucio Manzi, Ph.D., Associate Researcher — Merck Group, Guidonia, Italy


**AUTHORS**

L. Manzi, M. Sassi, M. Melchiorre, A. Palmese, and L. Colarusso
Pharmaceutical & Analytical Development Biotech Products, Merck Serono, Guidonia, Italy


**INDUSTRY**

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Merck is a leading science and technology company that operates across healthcare, life science, and performance materials.

**GENEDATA SOLUTION**

EXPRESSIONIST

# Enabling Novel MS-Based Methods for Characterization of Biopharmaceuticals

## Background

The Merck Serono structural characterization laboratory in Guidonia is part of the Characterization and Innovative Analytics Unit of the Analytical Development Biotech Department. Our role is to advise and support Merck worldwide in the development and production of novel biopharmaceuticals by providing a comprehensive structural overview of drug molecules and supporting process development and validation through advanced characterization approaches. The ability to deliver information-rich data at a molecular level makes mass spectrometry (MS) the technology of choice for applications such as (glyco)peptide mapping, disulfide-bridge and intact-mass analysis, top-down sequencing, and released glycan analysis. Our MS facility uses instruments from several different manufacturers, which are used on a case-by-case basis to perform a wide range of MS experiments. This case study presents the development and optimization of MS-based methods for charge-variant analysis (CVA) and glycosylation profiling and subsequent data processing using Genedata Expressionist, and describes the advantages over assays that had previously been performed using other technologies and data-processing approaches.

## Main Challenges

**Developing novel and efficient MS-based characterization methods**

Optimization of sample prep and separation procedures was the first challenge we addressed when developing novel MS-based analytical methods. However, to bring the overall efficiency of the end-to-end analytical process to the level required at Merck, we also required a platform that streamlined MS data processing, analysis, and management.

**Harmonizing analysis and preventing bottlenecks**

Acquiring data with instruments from different manufacturers means dealing with multiple software packages that create data silos and hinder comparison of results. In addition, the analysis of large volumes of complex data typically generated during MS experiments represents a serious bottleneck when using nonscalable desktop applications.

**Increasing data quality and confidence in results**

The large volumes of information-rich data generated during MS experiments require expert interpretation and dedicated application-specific algorithms to extract the maximum amount of information and deliver the most comprehensive characterization results possible. Where possible, error-prone manual evaluation (e.g., peak boundary determination) should be replaced by unbiased algorithms to improve result accuracy.

**Breaking down data barriers and improving knowledge sharing**

After data has been analyzed, the knowledge obtained should be freely accessible to all stakeholders to improve decision-making. Presenting complex information in a form that is quickly and readily understood is vital to the dissemination of knowledge within and across labs.

## Solution

**Optimized sample preparation and flexible, automated data processing**

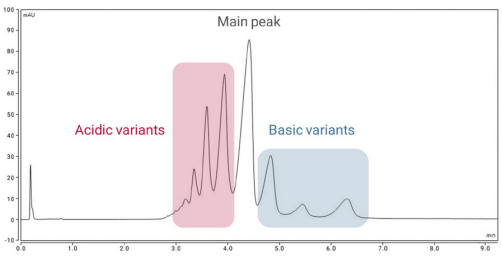
We developed novel sample preparation methods that used alternative chromatographic matrices, MS-compatible buffers, and reagents that generate analytes that are amenable to efficient MS analysis. During method development, the flexibility of Genedata Expressionist enabled us to comprehensively analyze results and iteratively optimize procedures. After method optimization was complete, the workflow-based system enabled us to automate data analysis, further streamlining the overall analytical process.

**Vendor-neutral, highly scalable data analytical system**

The ability of Genedata Expressionist to load data from any MS instrument enables us to process data from all our instruments using a single platform. As a centralized server-based enterprise solution, Genedata Expressionist scales to the needs of large organizations and allows us to quickly and efficiently analyze the large amounts of MS data that we generate.

**Streamlined MS analysis using custom libraries**

In CVA, we use the flexibility of Genedata Expressionist to perform two approaches within a single data processing workflow. In a targeted approach, custom molecular libraries containing entries corresponding to commonly encountered




| Mass | RT | Modifications | Glycosylation | Mass (1) | RT (1) | Mass Delta [ppm] | SCX_Erbitux_25ug |
|---|---|---|---|---|---|---|---|
| 153100.6 | 3.063 | 2*Gln->pyro-Glu + 2*Lys-loss | FA2 + FA2G1 + 2*FA2G2Sg2 | 153095.3 | 3.06 | 34.68 | 28.31 |
| 152950.8 | 3.408 | 2*Gln->pyro-Glu + 2*Lys-loss | FA2 + FA2G1 + FA2G2Ga1Sg1 + FA2G2Sg2 | 152950.2 | 3.41 | 3.97 | 19.83 |
| 152807.0 | 3.650 | 2*Gln->pyro-Glu + 2*Lys-loss | FA2 + FA2G1 + 2*FA2G2Ga1Sg1 : FA2 + FA2G1 + FA2G2Ga2 + FA2G2Sg2 | 152805.1 | 3.65 | 12.33 | 10.57 |
| 152659.6 | 3.974 | 2*Gln->pyro-Glu + 2*Lys-loss | FA2 + FA2G1 + FA2G2Ga1Sg1 + FA2G2Ga2 | 152660.0 | 3.97 | -2.72 | 9.59 |
| 152517.6 | 4.446 | 2*Gln->pyro-Glu + 2*Lys-loss | FA2 + FA2G1 + 2*FA2G2Ga2 | 152514.8 | 4.45 | 18.33 | 17.48 |
| 152642.2 | 4.888 | 2*Gln->pyro-Glu + Lys-loss | FA2 + FA2G1 + 2*FA2G2Ga2 | 152643.0 | 4.89 | -5.27 | 7.48 |
| 152911.3 | 5.525 | 2*Gln->pyro-Glu | FA2 + FA2G1 + FA2G2Ga1Sg1 + FA2G2Ga2 | 152916.3 | 5.53 | -32.63 | 6.55 |
| 152770.7 | 6.398 | 2*Gln->pyro-Glu | FA2 + FA2G1 + 2*FA2G2Ga2 | 152771.2 | 6.40 | -3.54 | 0.15 |

❶ *Upper panel: Elution profile of a UHPLC SCX separation of Cetuximab charge variants; Lower panel: Identification of principal species found in the targeted approach.*

species are used to provide an overview of highly abundant, expected variants across the entire elution profile (Figure 1). In an untargeted approach, more extensive libraries are used to fully characterize timeresolved elution ranges. Together with intuitive and insightful visualizations, the ability to search sample-specific custom libraries and perform advanced iterative review streamlines the analysis of released glycan and glycopeptide mapping results.

### Enterprise software with advanced reporting

We can fully integrate Genedata Expressionist into our existing data systems to create a knowledge database that allows existing product knowledge (for example, the nature of variants found in specific clusters) to be leveraged in subsequent analyses. Structured, highly configurable reports and visualizations provide at-a-glance interpretation that speeds up analysis and aids decision-making. For example, an XIC offset view of Fab subunit glycoforms demonstrates that they elute in order of increasing complexity, which aids their identification (Figure 2).

## Benefits

### Deeper characterization

Pairing the novel methods with an automated data analysis platform enabled us to maximize our labs' productivity and provided key insights into charge-variant and glycan analysis of biopharmaceuticals. For example, in middle-down glycosylation profiling, the novel sample preparation delivered a significant 46% increase in the number of identified glycoforms.

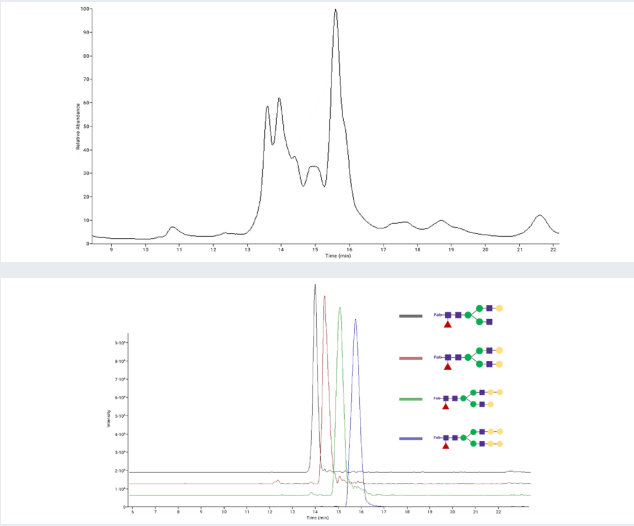
Using Genedata Expressionist for data analysis further boosted the total number of detected glycoforms with a striking 2.5-fold increase with respect to the conventional sample preparation and data processing methods—and minimized false-positives by using a custom-built glycan library.

### Harmonized processes and significant time savings

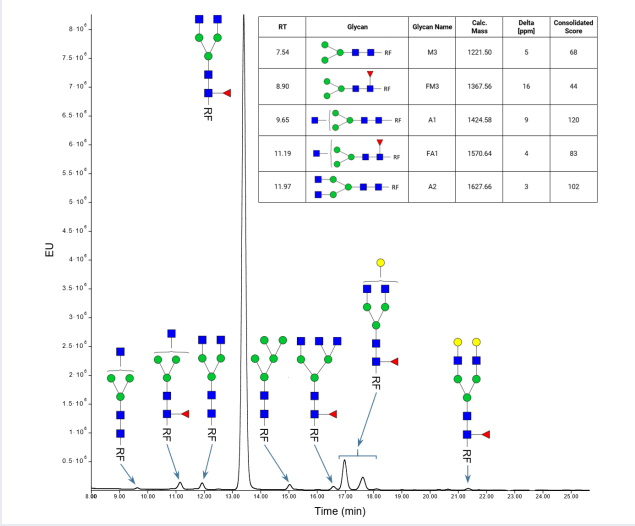
As a single-software solution for all MS-based applications, Genedata Expressionist breaks down data barriers and enables us to compare results from all our instruments using harmonized procedures. Because Genedata works closely together with leading instrument manufacturers, we can be sure that we can fully benefit from our investments in the latest MS technologies. Cutting-edge algorithms and the highly configurable workflow nature of Genedata Expressionist—which enables automated data processing and reporting—reduce the time required for data analysis by up to 80% compared to previously used methods (Figure 4). Automatic, unbiased integration and quantification eliminates the need for manual evaluation of signals.

### Higher-quality results

Using Genedata Expressionist not only provided significant time-savings but also increased the quality of results in all our analyses. Targeted MS analysis using custom libraries and consolidated scores provided high-confidence results and significantly reduced the number of false-positive identifications in CVA and glycosylation profiling following HILIC-MS analysis. In released glycan analysis—after their structure was assigned and confirmed by MS/MS analysis—glycans were automatically quantified based on their fluorescence detector signal, increasing the overall specificity of the method.



❷ ***Upper panel**: HILIC-MS TIC profile of WS 2014/01 digested with FabALACTICA; **Lower panel**: Extracted ion chromatogram profiles of Fab subunit glycoforms of increasing complexity*




❸ *Annotated FLR trace; **Inset**: Typical report table listing identified released glycans with consolidated MS/MS score.*

**Efficient dissemination and leverage of knowledge**
Reports generated using Genedata Expressionist can be configured by adding or removing individual elements to provide as much or as little detail as required. An overview report can be generated for quick comparison of samples within a batch, or a complete report detailing all results and data processing parameters can be created for analyses in regulated environments.

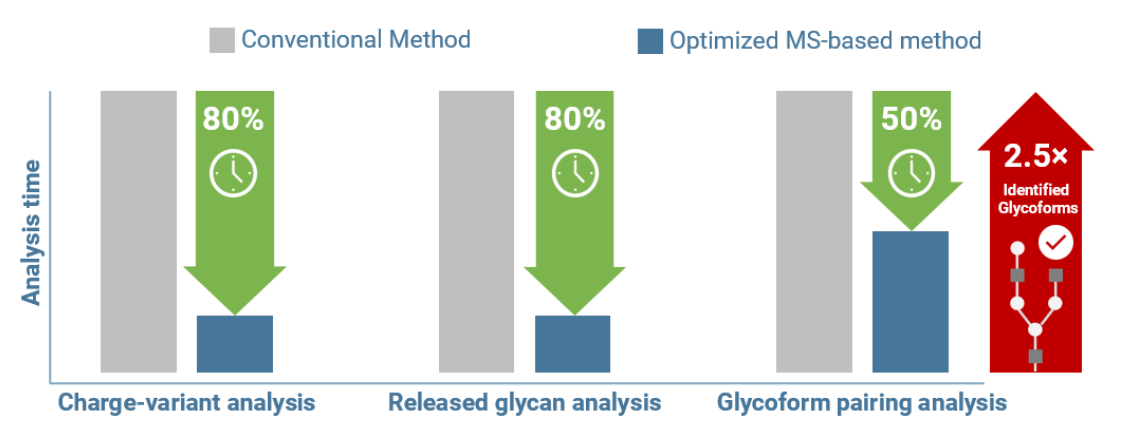
Curated results can be quickly leveraged to optimize subsequent analyses. For example, the custom-built library that reduced falsepositives in middle-down glycosylation profiling was derived from released glycans identified in previous experiments.
In a similar fashion, a subset of modifications found in a charge variant cluster were saved as a custom library to reduce combinatorial complexity and data processing time when analyzing corresponding clusters in subsequent experiments.

## Summary

Genedata Expressionist enabled us to rapidly develop novel methods and automated data workflows that were specifically customized and optimized for our novel MS-based biopharmaceutical characterization approaches. Utilizing a scalable platform allows us to rapidly process the large volumes of complex data that our labs generate—thereby increasing our overall productivity—and automated reporting facilitates collaboration and knowledge sharing across our organization.

## Outlook

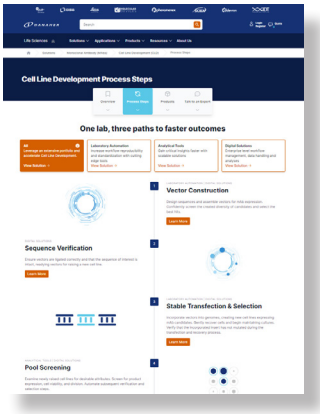
We are in the process of establishing a Good Manufacturing Practice (GMP) environment at our site. The compliance capabilities of Genedata Expressionist—which include detailed client and server logs, result review and approval, stringently defined projectspecific user/role profiles, and the ability to "lock down" optimized Genedata Expressionist workflows—will facilitate harmonized and reproducible MS data processing.




❹ *Time savings and information gains provided by novel protocols and Genedata Expressionist data processing.*

> ***Automated data processing and report generation provide remarkable time-savings in all our MS-based biopharmaceutical characterization applications.***
>
> Mauro Sassi, Ph.D., Associate Researcher — Merck Group, Guidonia, Italy

# Further Reading & References




Danaher Cell Line Development Capabilities

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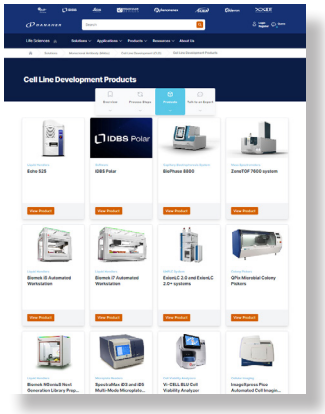


Cell Line Development Product Portfolio

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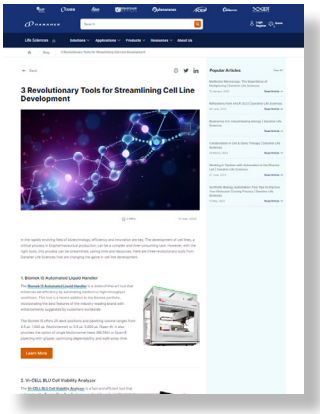


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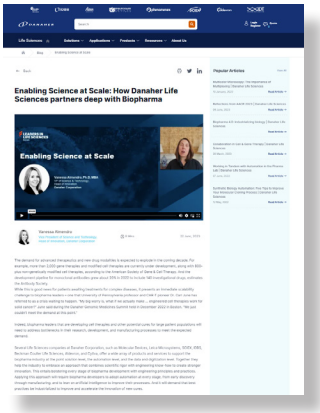


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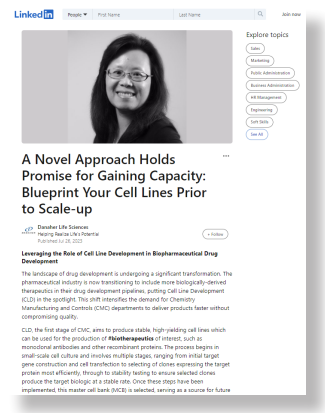


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We see a way to identify high producers in

<15

minutes in cell line development

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250x

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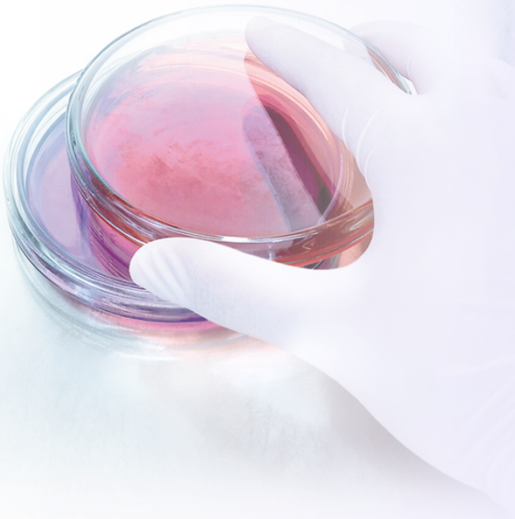

We see a way to save over

14,000

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