BECKMAN COULTER
Life Sciences

Automation of IgG Quantification using the Biomek i7 Hybrid Automated Workstation

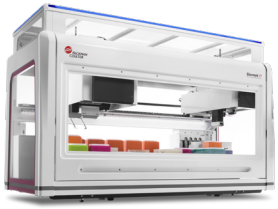
Beckman Coulter Life Sciences, Indianapolis, IN

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).



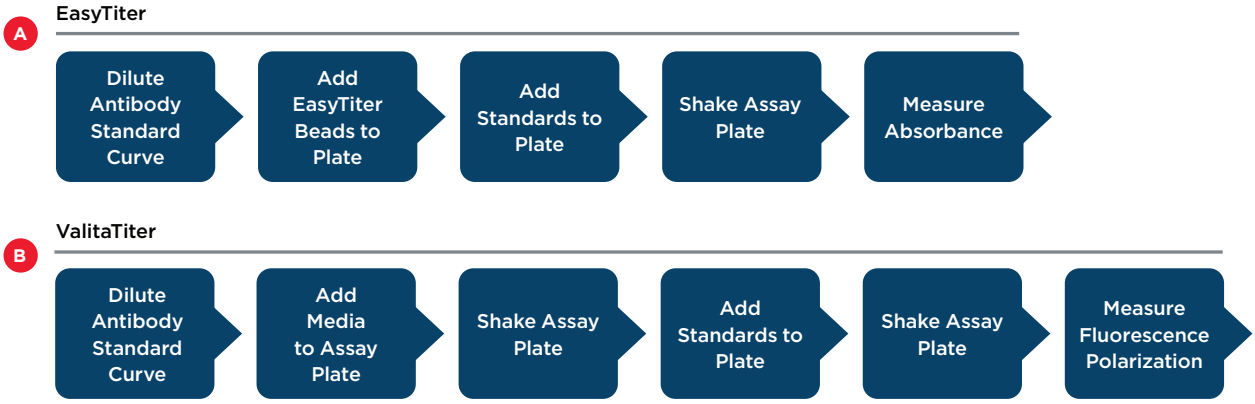


Figure 1. IgG Quantification Workflows. Flowcharts of ThermoFisher Easy-Titer **(A)** and ValitaCell ValitaTiter **(B)** IgG quantification assays. Steps performed using the automated Biomek method are highlighted in blue.

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.

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

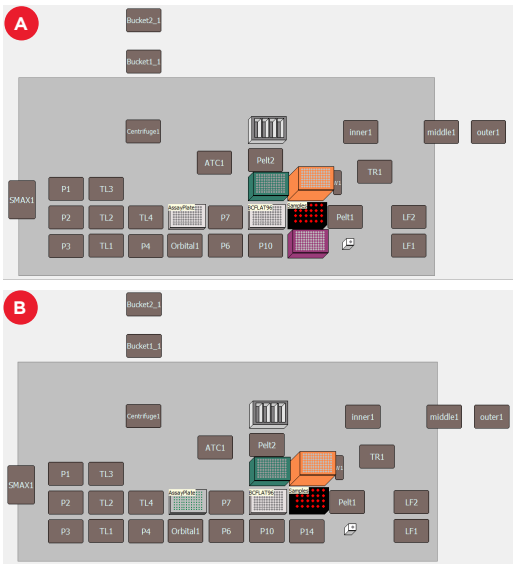


Figure 3. Biomek i7 Hybrid Deck Layouts. Deck layouts of automated ThermoFisher Easy-Titer **(A)** and ValitaCell ValitaTiter **(B)** IgG quantification assays. Together the automated method required the following deck components: 7 1X1 ALPs, Orbital Shaking ALP, and an integrated Molecular Devices SpectraMax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

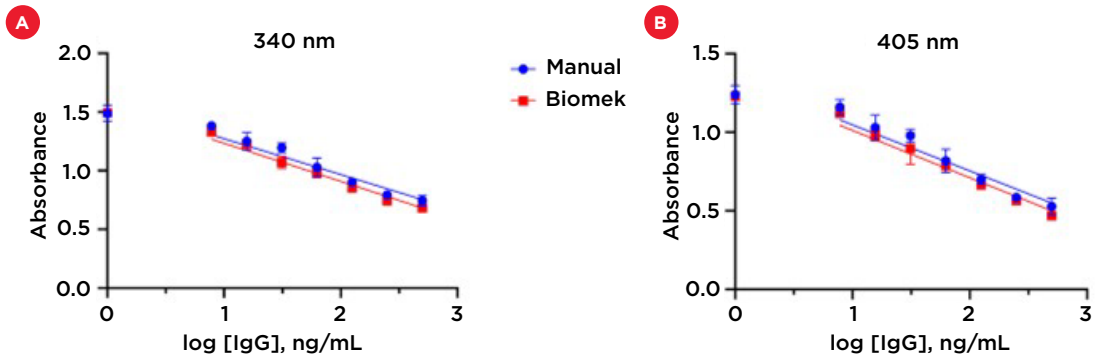


Figure 4. Easy-Titer Human IgG Standard Curve. Results of Biomek (Red) and manually (Blue) performed Easy-Titer assays. Absorbance was measured at 340 nm **(A)** and 405 nm **(B).** Data represent mean ± S.D. of n=3 replicate wells.

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.

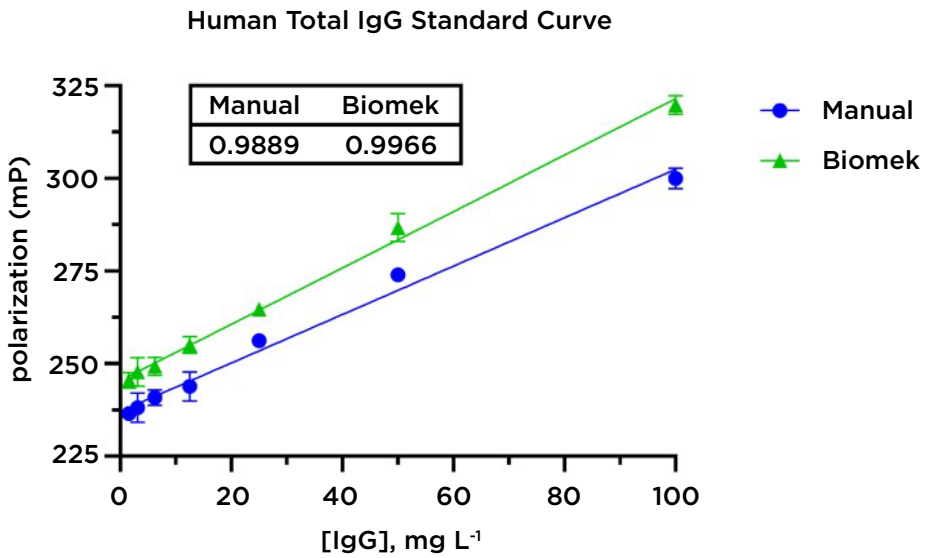


Figure 5. ValitaTiter Human IgG Standard Curve. Results of Biomek (Green) and manually (Blue) performed ValitaTiter fluorescence polarization assays. Data represent mean ± S.D. of n=3 replicate wells.

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.

References

1. Norman, D. J., Shield, C. F., 3rd, Barry, J., Henell, K., Funnell, M. B., & Lemon, J. (1987). A U.S. clinical study of Orthoclone OKT3 in renal transplantation. *Transplantation Proceedings,* 19(2 Suppl 1), 21–27.

2. Mullard A. (2021). FDA approves 100th monoclonal antibody product. *Nature Reviews Drug Discovery*, 20(7), 491–495. **https://doi.org/10.1038/d41573-021-00079-7**

3. Johnson D. E. (2018). Biotherapeutics: Challenges and Opportunities for Predictive Toxicology of Monoclonal Antibodies. *International Journal of Molecular Sciences,* 19(11), 3685. **https://doi.org/10.3390/ijms19113685**.

4. Dobson, P. D., Coss, K. P., Doherty, C., Clifford, J., Thompson, B., & James, D. C. (2020). Cell function profiling to assess clone stability. *Biotechnology and Bioengineering,* 117(7), 2295–2299. **https://doi.org/10.1002/bit.27336**.

5. Li, F., Vijayasankaran, N., Shen, A. Y., Kiss, R., & Amanullah, A. (2010). Cell culture processes for monoclonal antibody production. *mAbs,* 2(5), 466–479. **https://doi.org/10.4161/mabs.2.5.12720**.

6. Kowalski, M., Apiyo, D., Takkar, R., Jones-Roe, T. (2017). Automated Optimization of IgG Production in CHO Cells. **https://www.beckman.com**.

7. ThermoFisher Scientific. (2013). Instructions: Easy-Titer IgG Assay Kits. **https://assets.fishersci.com/TFS-Assets/LSG/manuals/MAN0011338_EasyTiter_IgG_Asy_UG.pdf**.

8. ValitaTiter. (2021). **https://valitacell.com/products-valita-titer/**

Materials

Equipment	Manufacturer
Biomek i7 hybrid automated liquid handler 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.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at **beckman.com**

21.09.2634.AUTO