Automated, Rapid & Reproducible Measurement of Immunoglobulin G using Opentrons OT-2 liquid handling robot and Valita®TITER and Valita®TITER Plus









Biologic drugs are the largest and fastest growing segment of the Pharmaceutical industry, with sales of €500bn and an annual growth of 8%.¹ Their ability to target diseases with high specificity results in improved safety and effectiveness. However, this increases the cost of manufacturing significantly over their predecessors.

Every manufacturing process for potential biologics begins with cell line development, whether for clinical trials or market launch.

During cell line selection, thousands of single cell clones are screened for optimal growth and Immunoglobulin G (lgG) production attributes to identify those best suited to manufacturing pipelines. This process requires accurate and reproducible methodologies to ensure successful outcomes.

Commonly used methods for IgG quantification require high-cost, specialist equipment or skilled personnel. Examples include High-Performance Liquid Chromatography (HPLC) and surface interferometry, or time-consuming assays such as Enzyme-Linked Immunosorbent Assay (ELISA). Although an ELISA is a wellestablished plate-based method for protein quantification, it is a lengthy multi-step process.

This article demonstrates the benefits of Valita®TITER assay range when combined with Opentrons OT-2 automation technology and software. When used alongside the Molecular Devices multimode iD5 plate reader, this platform provides a cost-effective reproducible solution for accurate IgG quantification throughout drug manufacturing. The data presented here shows the advantages of integrating bench-top automation into microtiter plate-based assay workflows.

Opentrons OT-2 Liquid Handler

The Opentrons OT-2 is a fully customisable, fast, precise and low-cost benchtop liquid handler with the flexibility to run any plate protocols. Equipped with 2 pipetting arms and 11 labware positions, large protocols can be completed without intervention.

Protocol development with the Opentrons Python API 2.0 offers good flexibility and control over experimental workflows. Alternatively, a graphical protocol designer allows quick generation of straight forward processes. The protocol used here, in addition to numerous others used at the University of Sheffield, are freely available for use on GitHub² and are fully customizable via Opentrons API. This is integrated with the flexible Labware Creator, to easily import custom equipment.

Valita®TITER and Valita®TITER Plus Assay Principle

Valita®TITER and Valita®TITER Plus are rapid, high-throughput assays; quantifying IgG-Fc interactions with a fluorescently labelled derivative of protein G via fluorescence polarization (FP).

FIGURE 1

Assay Schematic of Valita®TITER assay for IgG quantification using Fluorescence Polarization



Figure 1. Each well of the plate is pre-coated with a fluorescently labelled Fc-specific probe (1). An IgG sample binds to the probe (2). Binding is measured via fluorescence polarization and rotational diffusion (3).

FP effectively analyses changes in the size of molecules. "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 fluorophore-IgG binding can be detected using the degree of light de-polarization. When the fluorescently labelled 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 proportion) and perpendicular (depolarized portion) planes to the exciting light. The FP is expressed as a normalised difference of the two intensities, typically expressed in milli-polarization units (mP).

FIGURE 2

Assay Principle



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

Materials and Methods

GEN2 SINGLE-CHANNEL AVAILABLE RANGES

- Valita®TITER [Gen 2] Plus Assay kit;
- Valita®TITER [Gen 2] Assay Kit;
- Molecular Devices iD5 Multimode Plate reader;
- OT-2 Liquid Handler;
- Native Human lgG1 standard (BioRad, Product Code: 5172-9017);
- CD-CHO medium (GibcoTM, Catalog No. 10743);
- Trough 12-channel (Axygen, Product No. RES-MW12-HP-SI);
- Nunc 96-well U-bottom plate (Thermo Scientific[™], Catalog No. 168136);
- Optifit tips, 0.5-200µ (Sartorius, Catalog No. 790201)

METHODS

Human IgG standard was re-solubilised in Phosphate-Buffered Saline (PBS) to a concentration of $5.00 (\pm 0.5\%)$ mg/ ml as per the manufacturer's instructions. Further dilutions to working concentrations were carried out in CD-CHO media.

The OT-2 trough was loaded with CD-CHO media and Human IgG1 standard, at 200mg/L or 2000mg/L for the Valita®TITER and Valita®TITER Plus assays respectively. The protocol was performed using the 8-channel P300 pipetting arm by the OT-2 as follows:

- Media was added to columns 2-12, and IgG1 standard to column 1 of the sample plate.
- A serial dilution of the IgG standard was performed across columns 1-11 of the sample plate, resulting in 8 independent serial dilutions. Column 12 contains assay blanks.
- Media was added to the Valita®TITER or Valita®TITER Plus plates to reconstitute the probe.
- Samples were added to the Valita®TITER or Valita®TITER Plus plates and mixed thoroughly by pipetting.
- Plates were incubated at room temperature for 30 minutes and read on the iD5 plate reader using the appropriate FP method (outlined in Table 1 and 2).

The full python script for this work can be found on ${\rm GitHub.^3}$

The OT-2 took 15 minutes to prepare each 96-well plate for analysis and could perform up to 3 assays consecutively without user intervention.

TABLE 1

Instrument settings for ValitaTITER assay Fluorescence Polarization measurement on the iD5 reader.

SETTING	MOLECULAR DEVICES iD5
Measurement Mode	Fluorescence Polarization
Excitation	485 nm (adjustable bandwidth)
Emission	535 nm (adjustable bandwidth)
Gain	Low
G-factor	1.00
Attenuation	1 OD
Integration time (ms)	400
Read Height (mm)	4.66

The same experimental procedures as described were completed by an experienced human operator, defined as having completed at least 20 Valita®TITER or Valita®TITER Plus assays.

TABLE 2

Instrument settings for ValitaTITER Plus Assay Fluorescence Polarization measurement on the iD5 reader.

SETTING	MOLECULAR DEVICES ID5
Measurement Mode	Fluorescence Polarization
Excitation	485 nm (adjustable bandwidth)
Emission	535 nm (adjustable bandwidth)
Gain	Low
G-factor	1.00
Attenuation	3 OD
Integration time (ms)	400
Read Height (mm)	4.66

Results

An investigation was carried out into the technical reproducibility of replicate IgG standard curve samples prepared using the Opentrons OT-2 vs a human operator. These were analysed by Valita®TITER and Valita®TITER Plus assays. Technical reproducibility was determined by comparing the average standard deviation (StD) and coefficient of variation (%CV) obtained between replicate samples across three plates at varying IgG concentrations.

Figure 3 provides an overview of the OT-2 performance. The average StD and %CV of each concentration was calculated for each replicate plate. These were then averaged and plotted as the inter-plate average versus concentration of IgG.

The Valita®TITER (Figure 3a) StD and %CV weighted interplate averages (total average of individually averaged values) obtained were 0.95 mg/ml and 0.61% respectively. The Valita®TITER Plus (Figure 3b) weighted inter-plate averages were 1.48 mg/ml and 1.04% respectively. In comparison the weighted inter-plate average StD and %CV respectively for a human operator were 0.98 mg/ml and 0.66% for Valita®TITER, and 1.85 mg/ml and 1.30% for Valita®TITER Plus.

The performance of the Opentrons OT-2 and the human operator were directly compared in Figure 4. The change in Δ -StD and Δ -%CV represent the change in StD and %CV of the OT-2 dataset when normalised against the manual dataset, represented by the dotted line at y=0. The change in standard deviation (Δ -StD) and change in coefficient of variation (Δ -%CV) of the OT-2 in comparison with the human operator is shown for the Valita®TITER assay (Figure 4a) and Valita®TITER Plus assay (Figure 4b). The average inter-plate Δ -StD and Δ -%CV obtained was -0.04 mg/ml and -0.06% for Valita®TITER, and -0.37 mgl/ml and -0.26% for Valita®TITER, respectively.

FIGURE 3

Technical reproducibility of Valita®TITER (a) and Valita®TITER Plus (b) IgG quantification using Opentrons OT-2 and Molecular Devices iD5 plate reader



Figure 3. Investigation into the technical reproducibility of Valita®TITER (a) and Valita®TITER Plus (b) for IgG quantification when prepared by the Opentrons OT-2 liquid handling robot and analysed using Molecular Devices iD5 multimode plate reader. Reproducibility was determined by comparing the average StD and %CV obtained between replicate samples across three plates at varying IgG concentrations.

FIGURE 4

Performance Assessment of the Opentrons OT-2 Compared to an Experienced Human Operator for Valita®TITER (a) and Valita®TITER Plus (b)



Figure 4. IgG was quantified by analysis on the Molecular Devices iD5 multimode plate reader. The average StD and %CV between replicate samples was obtained across three plates at varying IgG concentrations. The dotted line at y=0 represents the StD or %CV of the manual assays. The Δ -StD and Δ -%CV represent the change in StD and %CV, calculated by the normalisation of the Opentrons OT-2 dataset against the manual dataset.

Discussion and Conclusion

The accurate and reproducible quantification of IgG is essential throughout drug discovery and development. The results presented here demonstrate that the addition of automation into experimental workflows can offer several key advantages such as increased workflow capacity, whilst maintaining or improving reproducibility and reliability of data.

From the presented data, it can be concluded that the Opentrons OT-2 liquid handling robot performs the Valita®TITER assays with the same consistency and precision as a human operator, with very similar StD and %CV values observed across the assay concentration range. Additionally, when performing the Valita®TITER Plus assay, the Opentrons OT-2 outperformed the human operator reducing both the StD and %CV in 80% of the data points along the standard curve.

Combining the Valita®TITER assay range with Opentrons OT-2 liquid handling robot provides a cost effective, reproducible solution to the accurate quantification of IgG throughout drug manufacturing, with the benefit of freeing up user time.

Abbreviations

ELISA	Enzyme-Linked Immunosorbent Assay
FP	Fluorescence Polarization
HPLC	High-Pressure Liquid Chromatography
lgG	Immunoglobulin G
PBS	Phosphate-Buffered Saline
mP	Milli-polarization Units
StD	Standard Deviation
Δ-StD	Change in Standard Deviation
%CV	Coefficient of Variation
Δ-%CV	Change in Coefficient of Variation

Citations

- 1. http://www.mckinsey.com/industries/ pharmaceuticals-and-medical-products/ourinsights/rapid-growth-in-biopharma
- 2, 3. https://github.com/OscarSwindley/ Opentrons_Protocols_API2.0

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