Improving the technical reproducibility of IgG quantification using Andrew+ for Valita®TITER and Valita®TITER Plus sample and plate preparation

Background

There is a growing reproducibility issue across the bioprocessing (particularly in the development of biologics) space with a 10-fold increase in the retraction of scientific publications. In theory, researchers should be able to re-create experiments, generate the same results, and arrive at the same conclusions, thus helping to validate and strengthen the original work. However, reality does not always meet these expectations. Recent research has reported that scientists are only able to reproduce published results in 11[1] percent or 25[2] percent of attempts, consequently, resources and time are wasted, and the credibility of scientific findings are put at risk.

Failures of reproducibility cannot be traced to a single cause, however, poor research practice and experimental design can be a major contributor to the production of non-reproducible results. Implementing standardised, traceable, automated approaches in combination with smart analytics into workflows could mitigate some of these issues.

Introduction

Biologic drugs is the fastest growing, and now the largest, segment of the Pharmaceutical industry with sales of €500bn and annual growth of 8% pa[3]. Their ability to target diseases very specifically makes them much more effective, and much safer medicines. It also makes them significantly more expensive than their predecessors to manufacture. The manufacture of every potential biologic drug, whether for a clinical trial or for market launch, begins with Cell Line Development (CLD). During this manufacturing process, thousands of individual cell clones need to be screened (e.g. IgG quantification) to identify the clone best suited to manufacture the target drug of interest. This screening process requires accurate and reproducible tools and methods in order to ensure success.

Here we present the Valita®TITER assay range combined with Andrew Alliance's automation technology and software (OneLab) which provides a cost effective, reproducible solution for accurate IgG quantification throughout drug manufacturing. The data presented here shows the advantages of integrating automated pipettes or bench-top automation into microtiter plate-based assay workflows.

Valita®TITER and Valita®TITER Plus Assay Principle

Valita®TITER and Valita®TITER Plus are rapid, high-throughput assays, which are based on the detection of IgG-Fc interactions with a fluorescently labelled derivative of protein G using fluorescence polarization (FP).

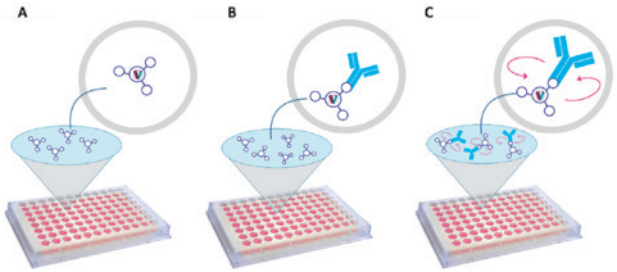


Figure 1: Assay Schematic of Valita®TITER Plus assay for IgG quantification using fluorescence polarization. Each well of the plate is pre-coated with a fluorescently labelled Fc-specific probe. An IgG sample binds to the probe (B). Binding is measured via fluorescence polarization and rotational diffusion (C).

FP effectively analyzes changes in the size of molecules. "Fixed" fluorophores that are excited by polarized light preferentially emit light in the same plane of polarization. However, rotation of the molecules between absorption and emission of the photon has the effect of "twisting" the polarization of the light. Small molecules tumble faster in solution than larger molecules. Hence, the change of size of molecules, with an associated fluorophore, can be measured using the degree of light de-polarization. Consequently, when the fluorescently labelled IgG-binding peptide is unbound, it tumbles rapidly and depolarizes the light more than when it is bound to an IgG (which is ~20 times larger). FP is measured by exciting the solution with plane polarized light and measuring the intensity

1. C.G. Begley, L.M. Ellis, "Drug development: Raise standards for preclinical cancer research," Nature, 483:531-33, 2012.
2. F. Prinz et al., "Believe it or not: How much can we rely on published data on potential drug targets?" Nat Rev Drug Discov, 10:712, 2011.
3. http://www.mckinsey.com/industries/pharmaceuticals-and-medical-products/our-insights/rapid-growth-in-biopharma

of light emitted in the plane parallel to the exciting light (polarized proportion) and perpendicular to the exciting light (depolarized portion). The FP is expressed as a normalized difference of these two intensities, which is typically in millipolarization units (mP).

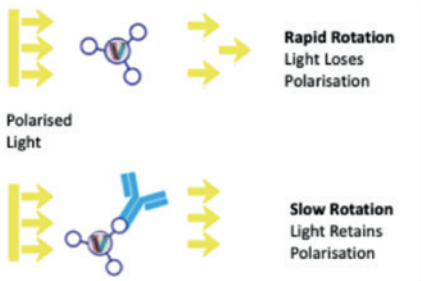


Figure 2: Assay Principle: The assay applies fluorescence polarization to quantify IgG. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

Improve the technical reproducibility of your IgG quantification using Andrew+ for Valita®TITER and Valita®TITER Plus sample and plate preparation

For the past number of years, scientists world-wide have been migrating towards integrated, automated workflows for drug discovery and screening platforms. The advantages of adopting high-throughput robotic platforms for screening a vast number of samples in an automated fashion are clear, however, for many experimental workflows, low-throughput, accurate benchtop automated solutions are the answer. An investigation was carried out in order to determine if the utilisation of Andrew Alliance's Andrew+ Robot to prepare both Valita®TITER and Valita®TITER Plus plates for analysis would increase the technical reproducibility of output data when compared to manual preparation. The following section provides an overview of the results obtained from this experiment.

Materials and Methods

Materials:

- Valita®TITER [Gen 2] Plus Assay kit;
- Valita®TITER [Gen 2] Assay Kit;
- BMG PHERAstar;
- Andrew+;
- OneLab Software;
- Human IgG1 standard (BioRad);
- Human IgG1 pre-quantified test; samples in spent cell culture media;
- Cell culture media [insert brand].

Method:

In order to determine if the utilisation of Andrew Alliance's Andrew+ robot to prepare both Valita®TITER and Valita®TITER Plus plates for analysis would increase the technical reproducibility of output, three plates of each assay type were prepared. One plate was prepared manually by an advanced user to both products, one plate was prepared manually by a new user to both products and one plate was prepared by the Andrew+ robot.

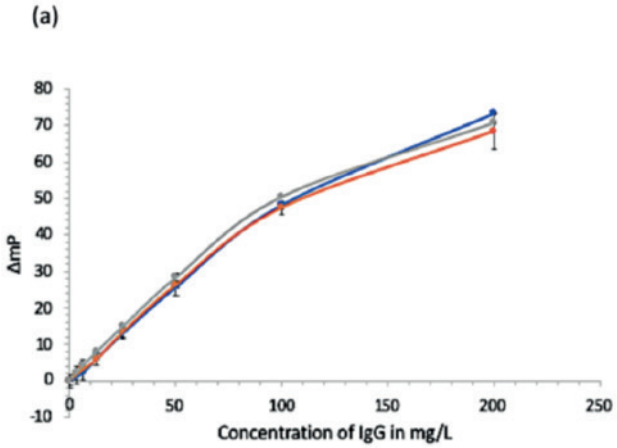
The measurement of 24 human IgG1 test samples was performed using both the Valita®TITER and Valita®TITER Plus 96-well plate-based assays and read using the BMG PHERAstar microplate reader. All samples and standards were prepared manually or using the Andrew+ robot according to the respective product instructions for use and the Valita®TITER and Valita®TITER Plus OneLab method where required.

Both assays were prepared in the same manner as described in section I method. Technical reproducibility was assessed by comparing the standard deviation between replicates for each test sample across each plate. The average standard deviation obtained for each plate set-up by either the advanced user manually, the new user manually or the Andrew+ robot were then compared to determine if the Andrew+ could aid in improving the technical reproducibility of output data for a new assay user.

Results:

Standard Curve Valita®TITER and Valita®TITER Plus:

An eight-point standard curve, prepared in triplicate, was analysed using Valita®TITER (0-200mg/L) and Valita®TITER Plus (0-2000mg/L).



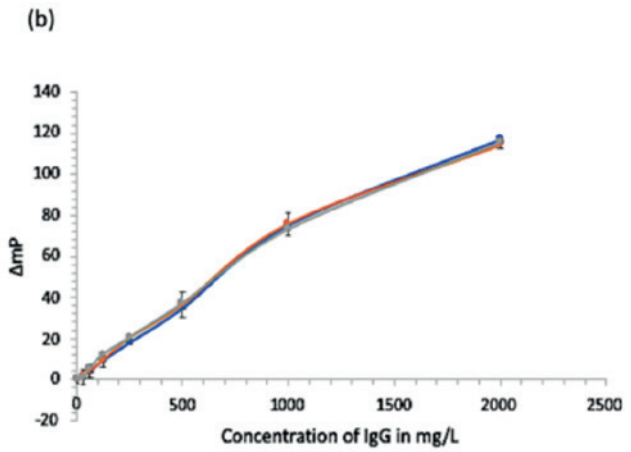


Figure 3: Human IgG standard curves prepared by different users manually versus the Andrew+ robot and analysed using Valita®TITER and Valita®TITER Plus. (a)

Three standard curves were prepared in the concentration range of 0-200mg/L (saturation). The first set of standards were prepared manually by an advanced user (blue), the second manually by a new user (orange) and the third was prepared using the Andrew+ robot (grey). Each standard was analysed on a Valita®TITER plate using fluorescence polarisation. The data was plotted in excel whereby the concentration of IgG in mg/L is plotted versus delta mP (zero subtracted); (b) Three standard curves were prepared in the concentration range of 0-2000mg/L. The first set of standards were prepared manually by an advanced user (blue), the second manually by a new user (orange) and the third was prepared using the Andrew+ robot (grey). Each standard was analysed on a Valita®TITER Plus plate using fluorescence polarisation. The data was plotted in excel whereby the concentration of IgG in mg/L is plotted versus delta mP (zero subtracted).

One set of standards was prepared by an advanced assay user manually (blue curve in Figure 3(a) (Valita®TITER) and (b) (Valita®TITER Plus)), the other by a new assay user manually (orange curve in Figure 3(a) (Valita®TITER) and (b) (Valita®TITER Plus)), and the last was prepared using the Andrew+ robot (grey curve in Figure 3(a) (Valita®TITER) and (b) (Valita®TITER Plus)).

The standard curve data obtained from both the advanced user and the Andrew+ robot, for Valita®TITER and Valita®TITER Plus, compared very well with standard deviations between triplicates <2. In contrast, similar to the data obtained from the Pipette+, the majority of standard deviations between triplicates for the new user were >2, with some >4. Variability between replicates in a standard curve for all relative quantification assays, like Valita®TITER and Valita®TITER Plus, will lead to the production of inaccurate and non-reproducible interpolation of the concentration of IgG for unknown test samples.

Investigation of technical reproducibility of the Andrew+ robot versus manual Valita®TITER and Valita®TITER Plus plate preparation:

An investigation into the technical reproducibility of pre-quantified test samples prepared using the Andrew+ robot versus manual preparation was carried out by a new assay user and an advanced assay user. Technical reproducibility was determined by comparing the average standard deviation obtained between triplicate test samples across a plate by each user (manually versus automated by the Andrew+ robot).

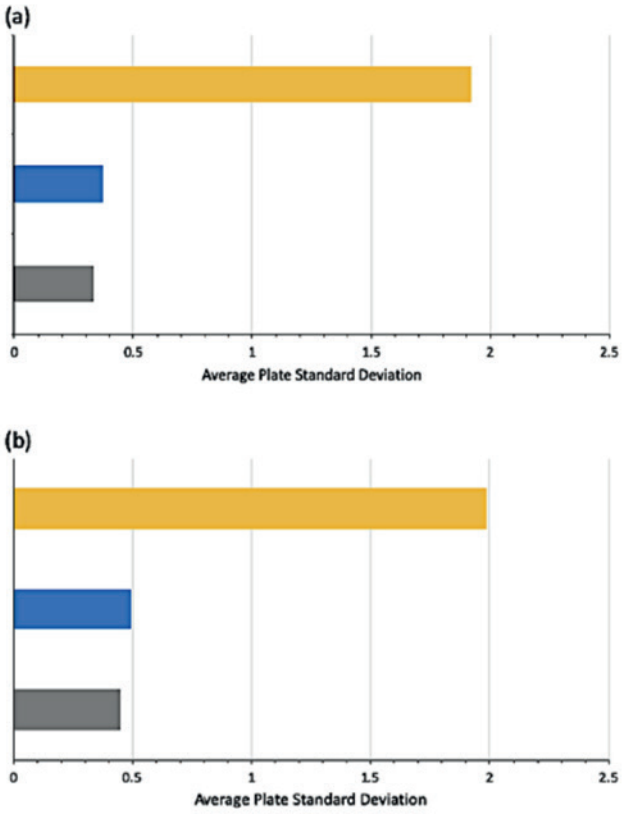


Figure 4: Investigation of technical reproducibility of the Andrew+ robot versus manual Valita®TITER (a) and Valita®TITER Plus (b) plate preparation (advanced user versus new user). A range of test samples were prepared for analysis following the standard Valita®TITER and Valita®TITER Plus sample preparation protocol. The first test sample set was prepared manually by a new assay user (orange), the second by an advanced user (blue) and the third set was prepared by the Andrew+ robot (grey). Each test sample set was prepared and analysed in triplicate using fluorescence polarisation. The average plate standard deviation obtained for each method by each user was calculated and plotted in excel

The data obtained from this experiment shows that the Andrew+ robot, combined with OneLab software, provides a simple, reproducible, cost-effective solution to automating the preparation of microtiter plate-based assays like Valita®TITER and Valita®TITER Plus for IgG quantification. By utilising this robot for assay plate preparation, the average standard deviation was reduced by ~78% for Valita®TITER and ~84% for Valita®TITER Plus.

Discussion and Conclusion:

The accurate and reproducible quantification of IgG is essential throughout drug discovery and development. The results presented here confirm that the addition of elements of automation into any experimental workflow has a number of key advantages including a reduction in human error and an increase in the production of reproducible, reliable data. Combining the Valita®TITER assay range with Andrew Alliance's automation technology and software provides a cost effective, reproducible solution to the accurate quantification of IgG throughout drug manufacturing.

Abbreviations

FP	Fluorescence Polarization
mP	Millipolarization Units

About the Authors

Dr. Hannah Byrne is the Head of Biological sciences at Valitacell Ltd. She studied Analytical Chemistry at Dublin City University and has a PhD in Biochemistry. Valitacell is a growing biotech company developing innovative technologies to aid and improve drug discovery and development.

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