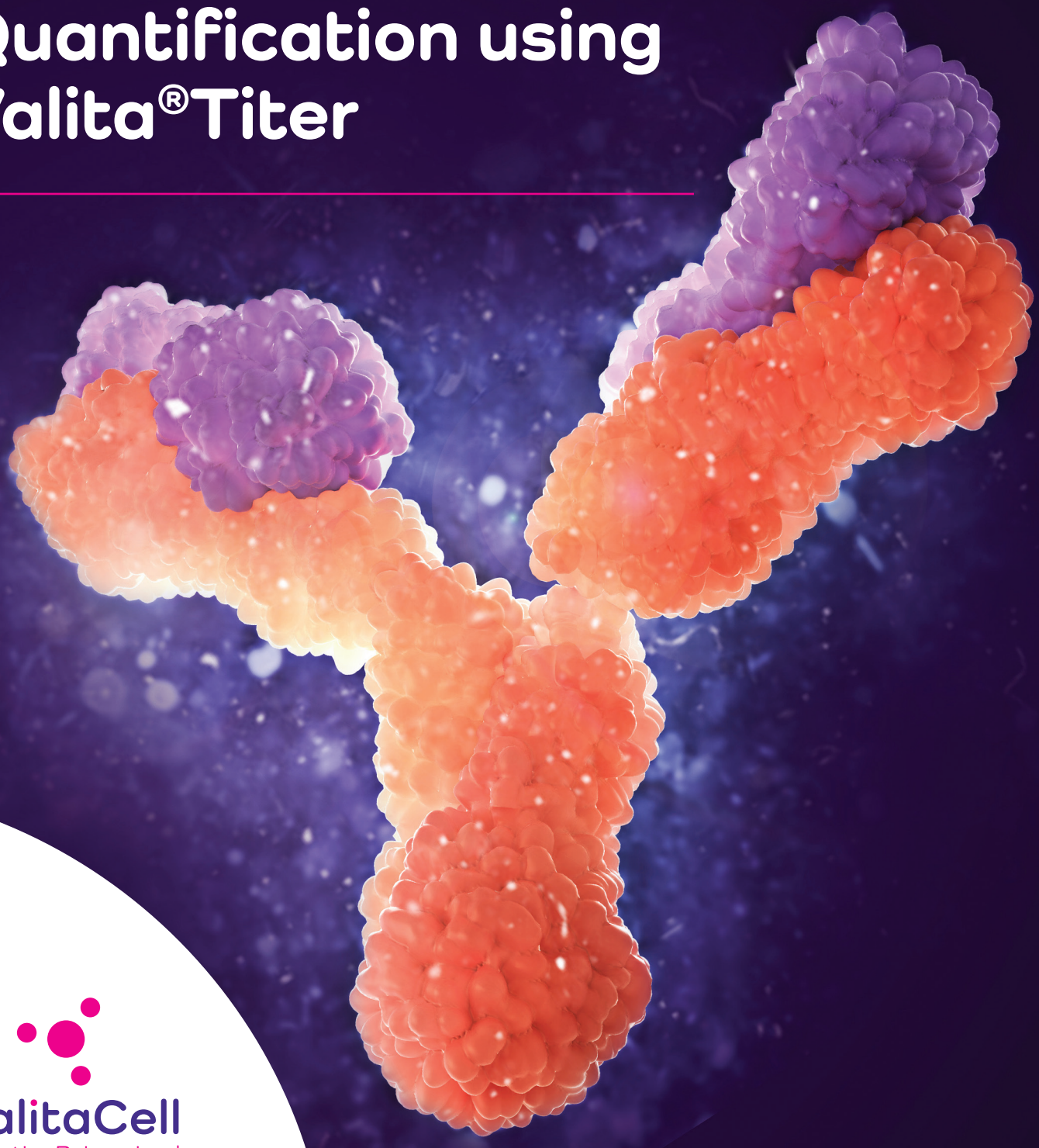
Application Note

Rapid Rabbit IgG Quantification using Valita®Titer



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Introduction

Rodents, such as mice and rats, have been the dominant host for antibody production since the discovery of hybridoma technology in the 1970s. However, as researchers expand their knowledge on the immune system of different species, other hosts have become attractive targets due to their unique characteristics. One such host are rabbits. In contrast to a rodent's immune system, rabbits seem to be able to recognize a much broader diversity of antigens. Additionally, antibodies produced by rabbits have exhibited a significantly higher affinity, specifically against epitopes of human origin, or epitopes known to be non-immunogenic in mice. Disadvantages of rabbits as hosts for antibody production are the increases in project time (due to longer immunisation regimens) and cost (due to lengthier and more complex husbandry requirements) when compared to rodent hosts. As such, the accurate, rapid, and cost-effective measurement of rabbit IgG throughout antibody development is essential and can aid in the mitigation of these disadvantages. This application note demonstrates the use of Valita®Titer as a high-throughput, rapid and precise tool for quantifying rabbit IgG.

Valita®Titer

Valita®Titer is a rapid, high throughput IgG quantification assay which uses fluorescence polarisation (FP) for detection. The assay relies on interactions between a fluorescently labelled IgG Fc-specific probe and the Fc of an IgG. FP effectively analyses changes in the size of molecules, given that smaller molecules tumble more rapidly than larger ones in solution. The rotation of the molecules between absorption and emission of the photon has the effect of 'twisting' the polarization of the light. 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). Therefore, FP is measured by exciting the solution with plane polarized light and measuring the intensity 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 millipolarisation units (mP).

Assay Principle

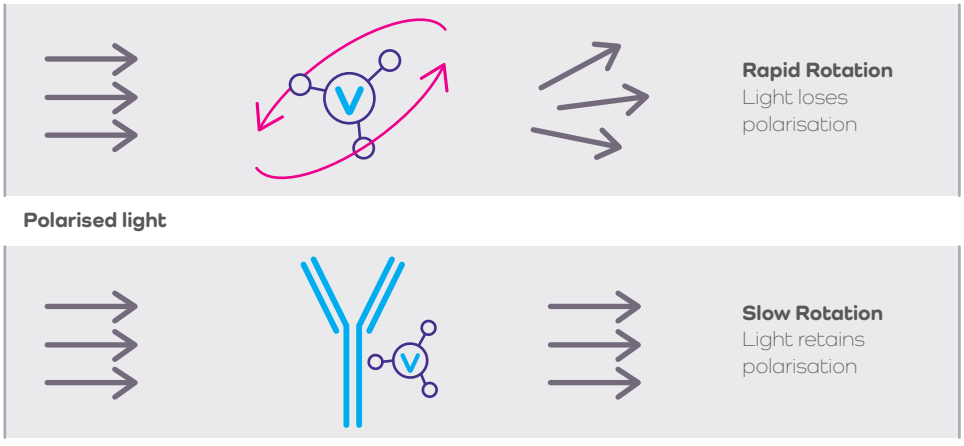

Figure 1
Valita®TITER assay principle. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

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Materials and Method

Materials

- Valita®Titer, catalogue number: VAL003, detection range: 2.5 - 100 mg/L
- CD CHO medium (GibcoTM, Catalogue No. 10743)
- BMG Labtech PHERAstar Multimode plate reader
- Monoclonal Rabbit IgG Isotype Control Antibody (LSBio, Catalogue No. LS-C742137)
- Normal Polyclonal Rabbit IgG Control (SinoBiological Catalogue No. CR1)
- ThermoFisher Finnpipette F2 Pipettes (Catalogue No. 10413865, 1187735, 11887351, 4662060)
- Starlab TipOne Tips (0.5- 200 µL Catalogue No. S1111-1700) (1000 µL Catalogue No. S1111-6811)

Method

BMG Labtech PHERAstar settings

Optic settings	Fluorescence Polarization, endpoint
	Optic module FP 485 520 520
	Focus and gain optimised from most fluorescent well (0 mg/L)
	70 mP target mP for gain
General settings	200 flashes per well
	0.5 s settling time

Monoclonal Rabbit IgG:

Monoclonal rabbit IgG standard was reconstituted in distilled H_2O to a concentration of 4 mg/mL as per the manufacturer's instructions. Dilutions were performed in CD-CHO media to prepare an 8-point standard curve ranging from 100 mg/L to 0 mg/L. Test samples were prepared from the 4 mg/mL stock solution diluted in CD-CHO media.

Polyclonal Rabbit IgG:

Polyclonal rabbit IgG was received from the manufacturers at a concentration of 1 mg/L in PBS pH 7.4. Serial dilutions were performed in CD-CHO media to prepare an 8-point standard curve ranging from 100 mg/L to 0 mg/L. Test samples were prepared from the 1 mg/mL stock solution diluted in CD-CHO media.

The assay protocol was performed using single and multichannel pipettes as follows:

Assay Procedure:

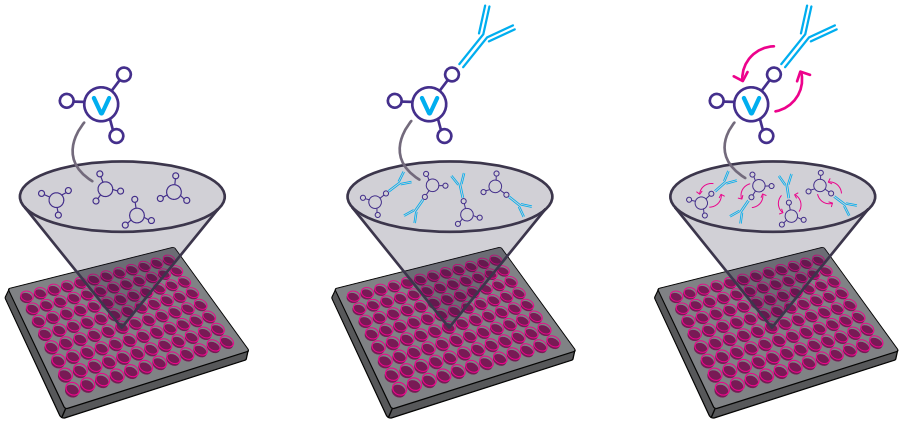
1. 60 µl of cell culture media was added to each well to reconstitute the Fc-specific probe (pre-dried onto the surface of the Valita®Titer assay plate)
2. 60 µl of each standard/sample was then added into appropriate wells of the 96-well plate
3. A multichannel pipette was used to mix each well 3 times prior to a 5-minute incubation in the dark
4. Post incubation, the plate was measured using FP

Figure 2
Assay Schematic of Valita®Titer assay for IgG quantification using fluorescence polarization.

1. Each well of the plate is pre-coated with a fluorescently labelled IgG Fc-specific probe

2. An IgG sample binds to the probe

3. Binding is measured using fluorescence polarization.



1. Valita®TITER plate is coated with an IgG specific binding peptide
2. IgG in test sample forms complex with the binding peptide
3. IgG concentration is measured using FP on a plate reader

Results

An investigation was carried out to assess the use of Valita®Titer to accurately detect and quantify rabbit IgG. Both monoclonal and polyclonal IgG molecules were investigated. As this is a relative quantification method, to enhance the accuracy of prediction, it is important to ensure that the molecule deployed as the standard, is homogeneous to the test samples. In this example, when quantifying mAb test samples, a mAb standard curve was used for interpolation. An eight-point standard curve (0 - 100 mg/L) for each molecule was prepared in duplicate and analysed using Valita®Titer. Alongside standard curve generation, samples of known concentration were interpolated against the standard curve and accuracy bias assessed.

A linear regression fit, whereby the concentration of IgG [mg/L] was plotted on the x-axis and Raw FP on the y-axis [Figure 3], was identified as the best fit for the mAb standard curve samples. The equation of the line was utilized to interpolate the concentration of mAb test samples by substituting the output Raw FP value for y and solving for x.

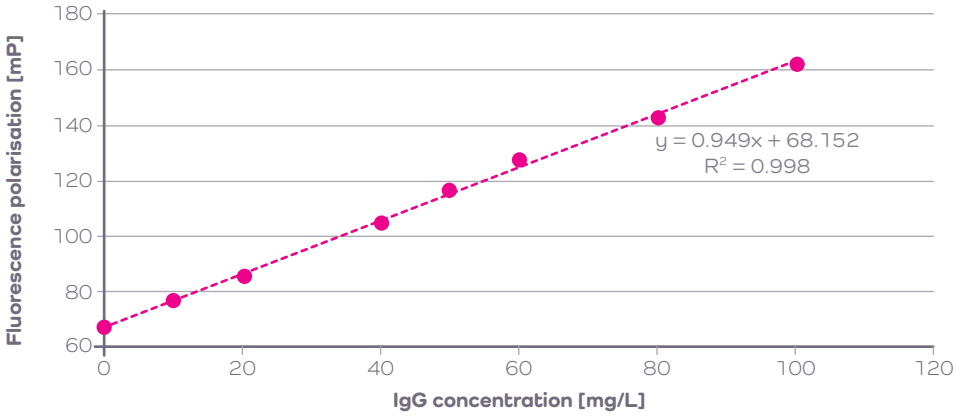


Figure 3
Rabbit monoclonal IgG Valita®Titer standard curve plotted using a linear fit (R2 = 0.998). Error bars show standard deviation of replicates.

Valita®Titer accurately interpolated the known concentration of test samples with a % accuracy bias versus the known absolute concentration of </= 8%, standard deviation of </= 2 mP, and an intra-plate co-efficient of variation of </= 2%, passing all typical acceptance criteria [Table 1]. Figure 4 demonstrates that output data generated using Valita®Titer correlates well with known absolute values, with an R^2 of 0.99.

Table 1: Summary of output data from the use of Valita®Titer for quantifying mAb test samples of known concentration.

Known IgG concentration in sample (mg/L)	Interpolated concentration rep1 (mg/L)	Interpolated concentration rep2 (mg/L)	Interpolated concentration rep3 (mg/L)	Average (mg/L)	Std. Deviation [mP]	Accuracy bias (%)	Coefficient of variation (%)
90	93.5	92.3	90.3	92.0	1.55	-2	0.999
60	64.3	63.8	61.1	63.0	1.63	-5	1.275
55	58.0	58.1	56.1	57.4	1.05	-4	0.856
40	39.7	39.0	38.8	38.8	0.92	+3	0.874
25	25.9	25.1	24.8	25.3	0.55	-1	0.596
12.5	13.3	13.6	13.4	13.5	0.14	-8	0.173
10	10.7	10.6	9.6	8.8	0.58	-3	0.745

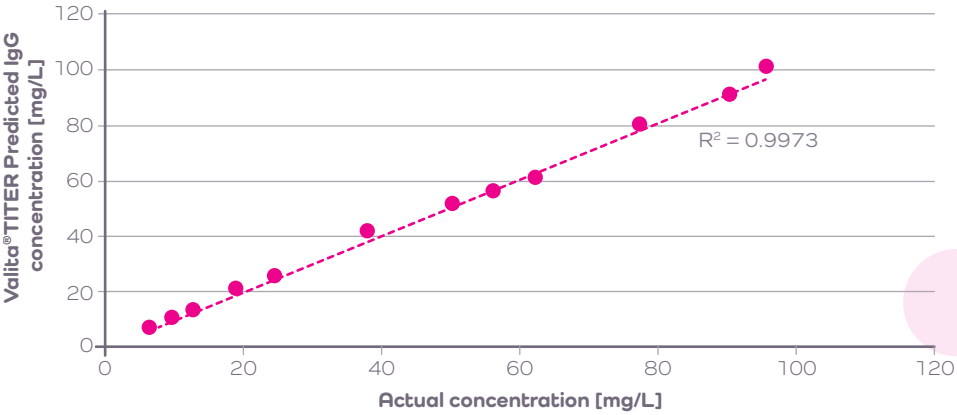


Figure 4
Valita®Titer predicted IgG concentrations plotted against actual concentrations for rabbit monoclonal IgG (R2 = 0.9973).

A 2nd order polynomial fit was identified as the optimum fit for the pAb standard curve samples. Here, the concentration of IgG [mg/L] is plotted on the y-axis and the Raw FP on the x-axis [Figure 5]. The equation of the line was utilized to interpolate the concentration of pAb test samples by substituting the output Raw FP value for x and solving for y. The assay accurately interpolated the known concentration of test samples with a % accuracy bias versus the known absolute concentration of </= 10%, standard deviation of </= 2 mP, and an intra-plate co-efficient of variation of </= 2%. [Table 2]. Figure 6 demonstrates that output data generated using Valita®Titer correlates well with absolute values, with an R^2 of 0.99.

Figure 5
Rabbit polyclonal IgG Valita®Titer standard curve plotted using a poly-2 fit (R2 = 0.9941). Error bars show standard deviation of replicates.

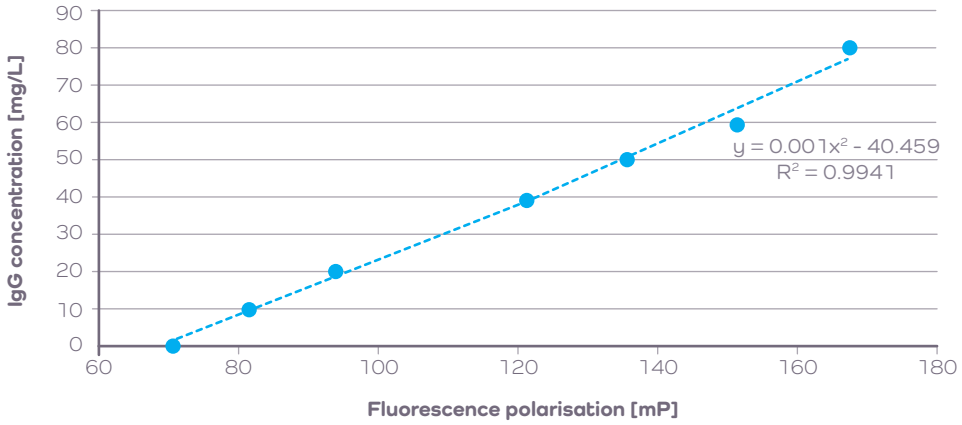


Table 2: Valita®Titer is capable of accurate interpolation of polyclonal rabbit IgG samples using a standard curve.

Known IgG concentration in sample (mg/L)	Interpolated concentration rep1 (mg/L)	Interpolated concentration rep2 (mg/L)	Interpolated concentration rep3 (mg/L)	Average (mg/L)	Std. Deviation [mP]	Accuracy bias (%)	Coefficient of variation (%)
90	84.1	84.1	83.9	84.1	0.11	+7	0.061
60	66.8	65.4	65.5	65.9	0.96	-10	0.623
55	57.4	56.2	55.5	56.4	1.15	-3	0.809
40	39.5	39.5	40.1	39.7	0.4	+1	0.331
25	24.1	24.2	24	24.1	0.13	+4	0.133
12.5	11.5	12.0	11.4	11.6	0.45	+7	0.54
10	9.0	9.6	10.0	9.5	0.71	+5	0.882

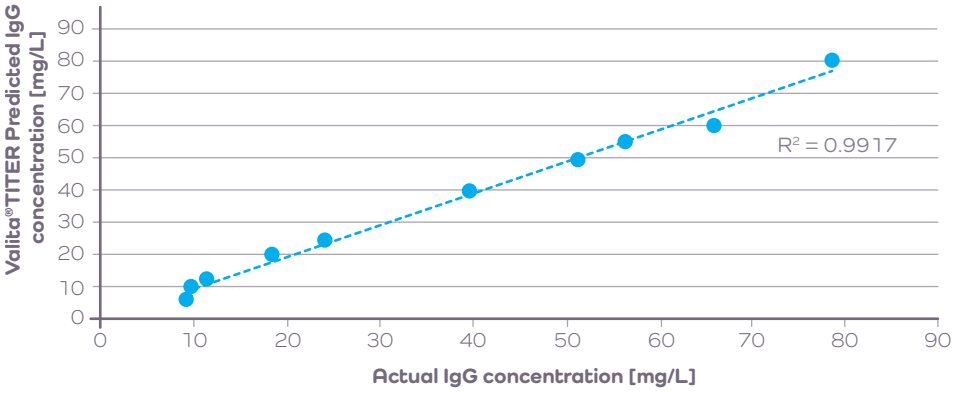


Figure 6
Valita®Titer predicted IgG concentrations plotted against actual concentrations for rabbit polyclonal IgG (R2 = 0.9917).

Conclusions

Valita®Titer is capable of robust, accurate measurement of rabbit IgG standards and samples. The assay allows for high-throughput, direct, rapid and precise quantification of crude rabbit IgG samples at various stages of development.

Abbreviations

FP Fluorescence polarization

mP Millipolarisation units

IgG Immunoglobulin G

About the Authors

Dr. Anna Boland is a Product Development Scientist at Valitacell Ltd. She studied Molecular Medicine at Trinity College Dublin and has a PhD in Medicine from Queen's University Belfast.

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