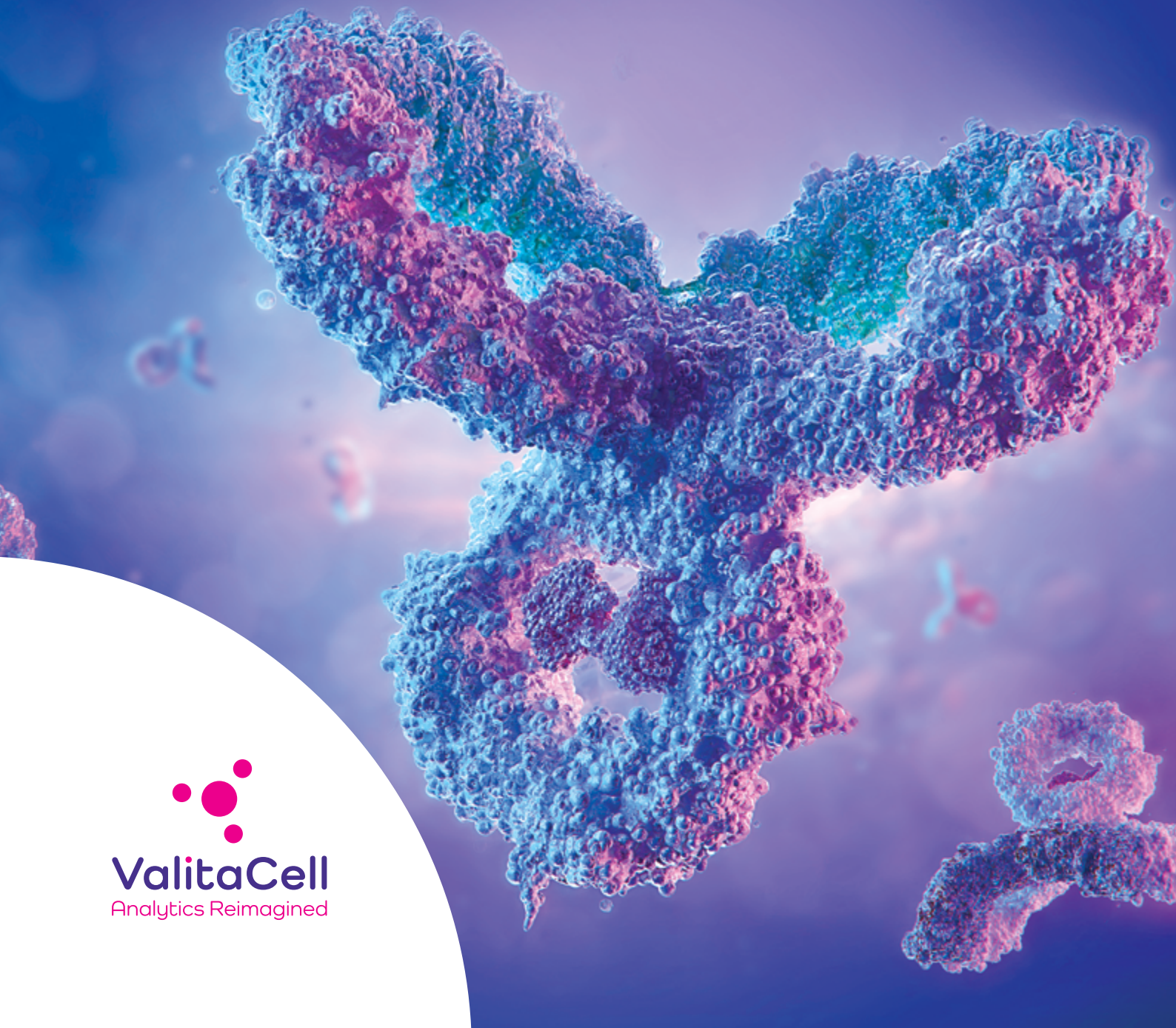
Technical Note

Rapid Canine IgG Quantification using Valita®Titer



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Introduction

Since the debut of Kohler and Milstein's hybridoma technology in 1975, it has been possible to generate vast quantities of pure monoclonal antibodies. Cut to present day, and therapeutic monoclonal antibodies (mABs) have emerged as the most common type of novel medicine developed in recent years (Lu et al., 2020).

Since December 2019, the FDA has authorized 79 mABs. These can be used to treat a variety of diseases including cancers, autoimmune, metabolic and infectious diseases (Lu et al., 2020).

The growing success of antibody therapies in humans has spurred an interest in developing similar therapies for dogs. For example, cancer will affect 6 million of the 70 million canines in the United States alone, and with limited therapies, there has been significant interest for more effective therapies (Klingemann, 2018).

Valita®Titer has already been developed and validated for quantification of Human IgGs with remarkable success, so the aim of this technical note is to investigate how the product could be integrated into a canine IgG screening campaign, to facilitate high-throughput, low-cost IgG quantification and relative ranking for potential canine IgG therapies.

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, Fc-specific probe, and the Fc-region of an IgG. FP effectively analyzes 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). FP is expressed as a normalized difference of these two intensities, which is typically in millipolarization units (mP).

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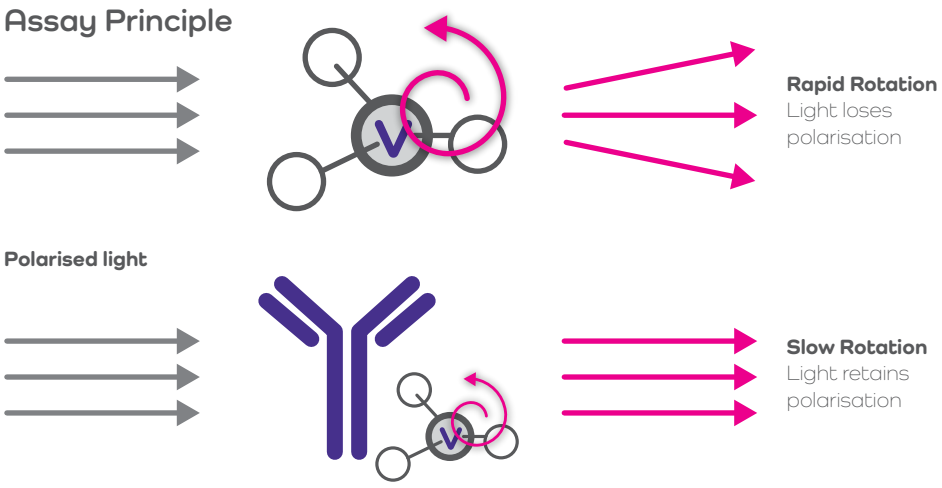


Figure 1
Valita®Titer assay principle. Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

Materials and Method

Materials

- Valita®Titer, catalogue number: VAL003, detection range: 40 – 400 mg/L (canine IgG, relative ranking)
- Native Dog IgG protein, 5 mg (Abcam, Catalogue no. AB198651)
- CD CHO medium (Gibco™, Catalogue No. 10743).
- Valita®Mab Buffer (ValitaCell™, Reference 500089, Lot 19014)
- BMG Labtech PHERAstar Multimode plate reader.
- 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

Canine IgG

Polyclonal Canine IgG was reconstituted in distilled H_2O to a concentration of 5 mg/mL, as per the manufacturer's instructions. Serial dilutions were performed in CD CHO media to prepare an 8-point standard curve from 400 to 0 mg/L. Test samples were also prepared from the 5 mg/mL stock solution diluted in CD CHO media.

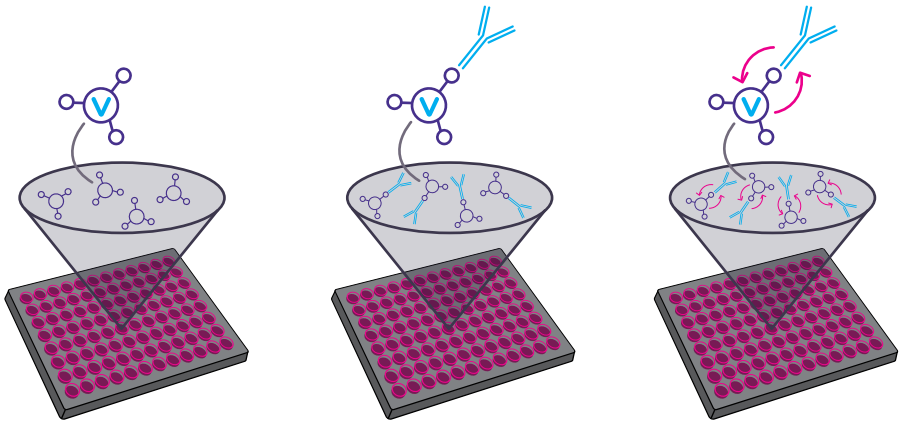
The assay protocol was performed using single and multichannel pipettes as follows:

Assay Procedure

1. 60 µL of Valita®MAb buffer was added to each well to reconstitute the IgG-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 5 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 ValitaTiter for quantifying canine IgG. As this is a relative quantification technique, a standard curve of known concentration was deployed alongside a range of pre-quantified test samples, analysed in triplicate. To improve the accuracy of test sample prediction, a molecule-specific standard curve was deployed here. Assay performance was assessed based on an accuracy bias for prediction of </=10% and a %CV of </=2%.

Data analysis was carried out using GraphPad (PRISM 9). A cubic fit (third order polynomial) with the Raw FP [mP] on the x-axis and the IgG concentration [mg/L] on the y-axis was determined to be the best fit for the Canine IgG standard curve (Figure 3). The equation of the line was used to interpolate the canine IgG test samples by substituting the output Raw FP for x and solving of y.

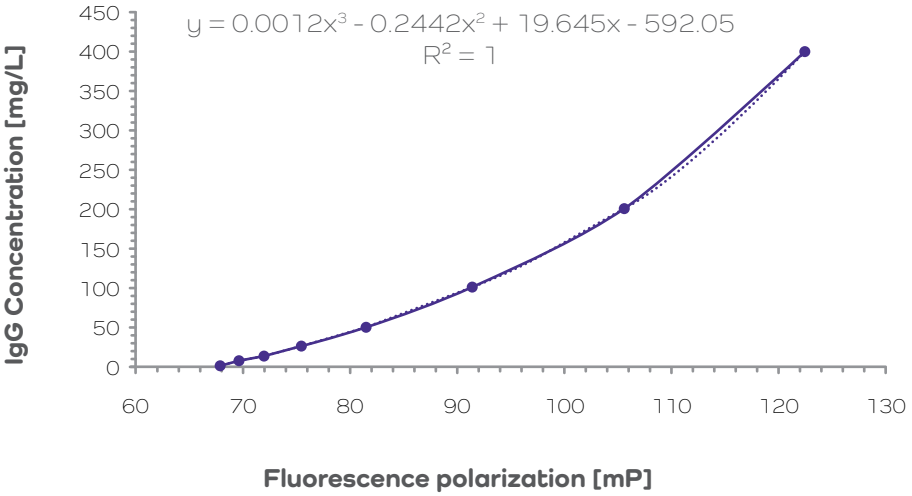


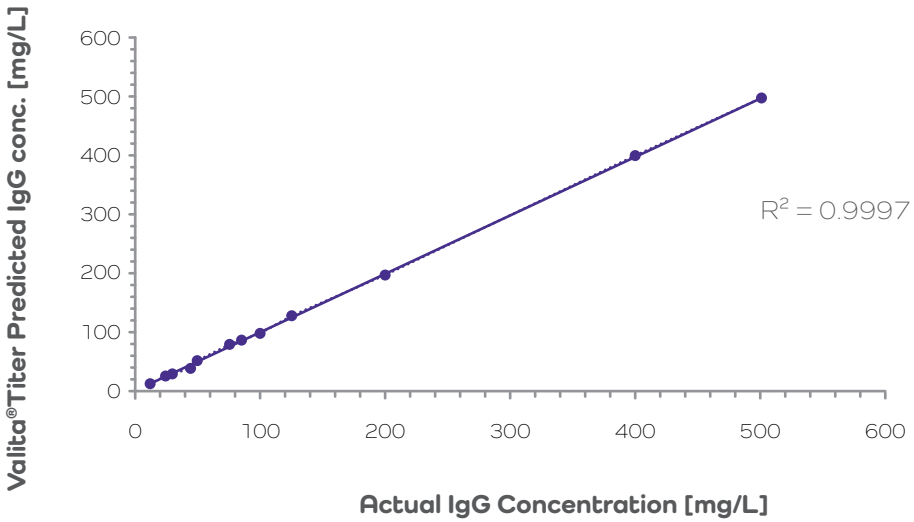
Figure 3
Canine IgG Valita®Titer standard curve plotted using a Cubic fit (poly-3 fit) (R2= 1).

Valita®Titer accurately interpolated the known concentration of test samples with a %bias versus the known absolute concentration of </= 10%, standard deviation of </= 2 mP, and an intra-plate co-efficient of variation of </= 2%, passing all typical acceptance criteria. Figure 4 demonstrates that output data generated using Valita®Titer correlates well with known absolute values, with an R2 of 0.99.

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

Known IgG Concentration	Average Raw FP (mP)	Standard Deviation	Interpolated concentration (mg/L)	Diff (mg/L)	Accuracy bias (%)	Coefficient of variation (%)
500	131.416476	1.05544284	499.2909787	0.70902133	-0.141804267	0.00803128
400	122.42841	1.84026765	402.4784182	-2.4784182	0.619604544	0.01503138
200	105.566748	1.22260118	197.6168559	2.38314409	-1.191572044	0.01158131
100	91.4088637	0.95332255	99.53352547	0.46647453	-0.466474528	0.01042921
50	81.5171952	0.59579703	51.3083046	-1.3083046	2.616609202	0.00730885
25	75.4864413	0.69378594	26.48525931	-1.4852593	5.941037237	0.00919087
12.5	72.0312123	1.33006061	13.42342247	-0.9234225	7.387379765	0.01846506
125	96.3104404	1.36880424	128.3829889	-3.3829889	2.706391103	0.01421242
85	88.9647691	1.59669214	86.54222496	-1.542225	1.81438231	0.01794747
75	87.8864767	1.61337888	81.06049595	-6.0604959	8.08066126	0.01835753
45	79.1657281	0.80292111	41.29198106	3.70801894	-8.24004208	0.01014228
30	76.3347978	0.94803404	29.81237742	0.18762258	-0.625408601	0.01241942

Figure 4
Valita®Titer predicted IgG concentrations plotted against the concentrations for canine polyclonal IgG (R2 = 0.9997).



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Conclusions

We have demonstrated the application of Valita®Titer for accurately quantifying canine IgG. The assay could be deployed at any stage during canine IgG manufacturing, facilitating high-throughput IgG screening, as well as facilitating faster run times and being a highly robust assay when working with cells.

A cubic fit is the optimum fit for canine IgG showing the best accuracy bias percentage and ValitaMab buffer for reconstitution showed an optimal delta shift, it is therefore recommended that both are utilized the obtain desirable results.

Abbreviations

FP Fluorescence polarization

mP Millipolarization units

IgG Immunoglobulin G

About the Authors

Shannon Slattery, MSc is a R&D Technical Application Specialist at Valitacell Ltd. She studied a BSc in Industrial Biochemistry, and a research MSc in Microbiology and Molecular Biology, both from the University of Limerick, Ireland.

Dr. Hannah Byrne is the Head of Biological Sciences at Valitacell Ltd. She studied Analytical Science 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.

References

Lu, R., Hwang, Y., Liu, I., Lee, C., Tsai, H., Li, H. and Wu, H., 2020. Development of therapeutic antibodies for the treatment of diseases. Journal of Biomedical Science, 27(1).

Klingemann, H., 2018. Immunotherapy for Dogs: Running Behind Humans. Frontiers in Immunology, 9.

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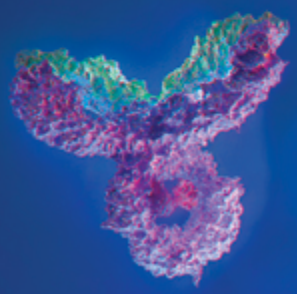

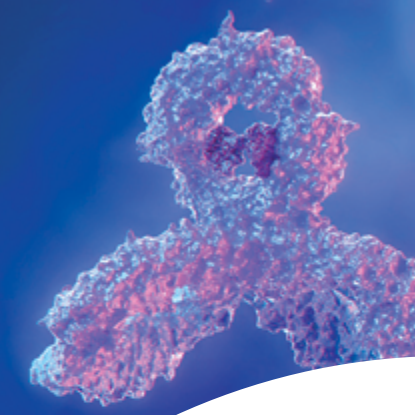

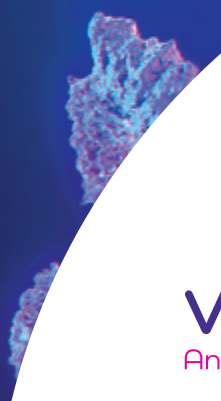

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