Improved data quality of plate-based IgG quantification using Spark®'s enhanced optics.

Technical Note

COMPARISON OF THE SPARK READER'S STANDARD AND ENHANCED FLUORESCENCE OPTICS FOR IGG QUANTIFICATION USING THE VALITATITER ASSAY.

INTRODUCTION

Florescence polarization (FP) can be used to effectively analyze changes in the size of molecules, as smaller molecules tumble more rapidly in solution than larger ones. The rotation of the molecules between absorption and emission of the photon has the effect of 'twisting' the polarization of the light. FP is measured by exciting the solution with polarized light and measuring the intensity of light emitted in both the parallel (polarized) and perpendicular (depolarized) planes, and is expressed as a normalized difference of these two intensities, typically in millipolarization units (mP).

The ValitaTiter and ValitaTiter Plus assays are rapid, high throughput FP assays for the detection of IgG antibodies with a fluorescently-labeled protein G derivative. These assays exploit the fact that the unbound fluorescently-labeled marker tumbles more rapidly and depolarizes the light more than when it is bound to an IgG, which is around 20 times larger (Figure 1).

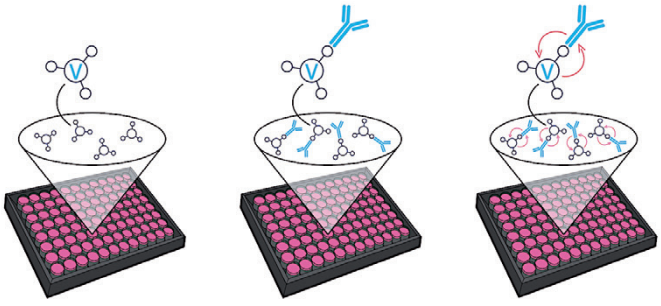

Figure 1: Assay schematic of ValitaTiter Plus assay for IgG quantification using FP. Each well of the plate is pre-coated with a fluorescently-labeled IgG-specific probe (1). IgG in a sample binds to the probe (2). Binding is measured via FP and rotational diffusion (3).

The choice of plate reader, optics and measurement settings all significantly affect the quality of the data for FP assays. For ValitaTiter assays, the power and frequency of the flash lamp used for sample excitation are critical, together with the choice of optical system used for wavelength selection; filter optics are more sensitive but less flexible, whereas monochromators are more flexible but less sensitive. The Spark multimode microplate reader offers FP with filters, monochromators or Fusion Optics that combine filters and monochromators in the same measurement. Additionally, the platform is available with standard optics – using a 5 W, 50 Hz xenon flash lamp – or with enhanced optics, using a 20 W, 100 Hz xenon flash lamp.

The power and the frequency of the flash lamp are very important for the throughput of a reader, as these determine the number of flashes and the measurement time required to obtain the desired data quality. The modular concept of the Spark allows users to combine different optical components to match their throughput, sensitivity and flexibility requirements. It also allows the addition of other read modes – including fluorescence intensity, absorbance, luminescence or brightfield imaging – making the Spark a multifunctional instrument that can support many lab assays.

This technical note outlines the use of the Spark reader in combination with the ValitaTiter assay for the quantification of IgG. It offers a comparison of the use of the instrument's standard and enhanced fluorescence optics, and discusses the optimization of flash number to produce high quality, reproducible data while minimizing read times.

MATERIALS AND METHOD

Materials

- 2 x ValitaTiter (Gen 2) assay plates
- Spark multimode microplate reader with standard optics
- Spark multimode microplate reader with enhanced optics
- Native human IgG standard (#5172-9017, BioRad)
- Gibco® CD CHO medium (#10743, ThermoFisher)

Method

Native human IgG standard was reconstituted in PBS to a concentration of 5 mg/ml (±0.5 %) as per the manufacturer's instructions. Serial dilutions were then performed in CD CHO media to prepare an 8-point standard curve ranging from 0 mg/l to 200 mg/l.
The assay protocol was performed using single and multichannel pipettes as follows:

1. 60 µl of cell culture media was added to each well to reconstitute the IgG-specific probe (pre-dried onto the surface of the ValitaTiter assay plate).
2. 60 µl of each standard was then added into appropriate wells and run in replicates across a 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. Each plate was then read using the two Spark instruments (individual instrument settings are outlined in Table 1 and Table 2, respectively).

5. Each plate was read 6 times, with the only variable being the flash number. The gain and z-position were kept constant from the first read to allow plate-to-plate comparability.

Parameter	Setting
Mode	FP
Excitation wavelength	485 nm
Excitation bandwidth	20 nm
Emission wavelength	535 nm
Emission bandwidth	20 nm
Flash number	30-100 range
Integration	40 µsec
Gain	Manual

Table 1: Instrument settings for ValitaTiter assay measurement on the Spark with standard monochromator optics.

Parameter	Setting
Mode	FP
Excitation wavelength	485 nm
Excitation bandwidth	20 nm
Emission wavelength	535 nm
Emission bandwidth	20 nm
Flash number	30-100 range
Integration	40 µsec
Gain	Manual

Table 2: Instrument settings for ValitaTiter assay measurement on the Spark with enhanced monochromator optics.

RESULTS

An investigation into the effect of flash number on the reproducibility and assay read time of replicate IgG standard curves was carried out, comparing Spark's standard monochromator optics with the enhanced monochromator optics. Reproducibility was determined by comparing the average standard deviation (SD) obtained between replicate samples across an entire 96-well plate, at varying IgG concentrations, for each flash number (30, 40, 50, 60, 80 and 100 flashes). Figure 2 provides an overview of the data, where the average SD is plotted against IgG concentration (in mg/l) for each flash number.

It is clear from the results that, when using the standard optics (Figure 2a), a high flash number (>80 flashes) is required to reduce the SD between replicate samples to an acceptable range (SD <2). This increases the read time and, therefore, the overall data acquisition time (Figure 3). In contrast, the enhanced optics reduce the need for a high flash number in order to obtain reproducible data (Figure 2b). Using this set-up, 30 flashes (the minimum tested) led to the production of acceptable data in a fraction of the time (Figure 3).

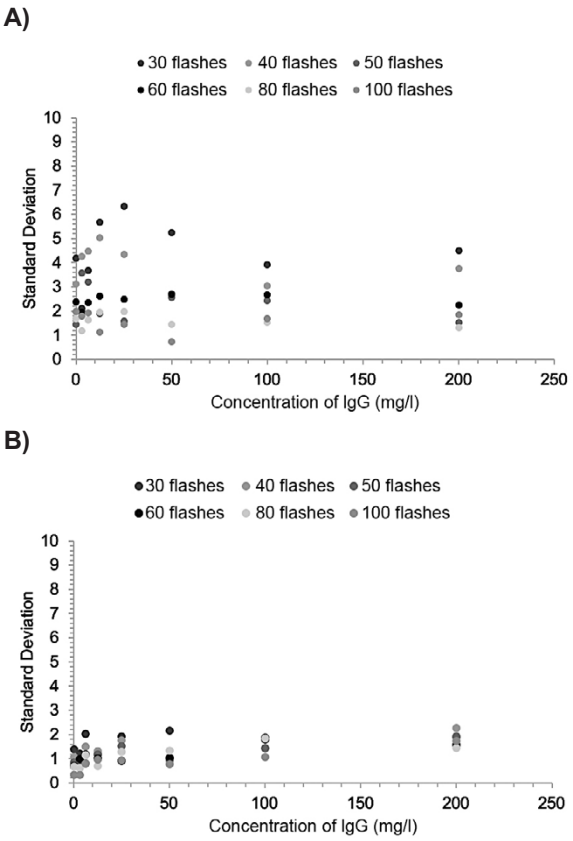

Figure 2: Scatter plots of showing impact of flash number on standard deviations with (a) standard optics and (b) enhanced optics.

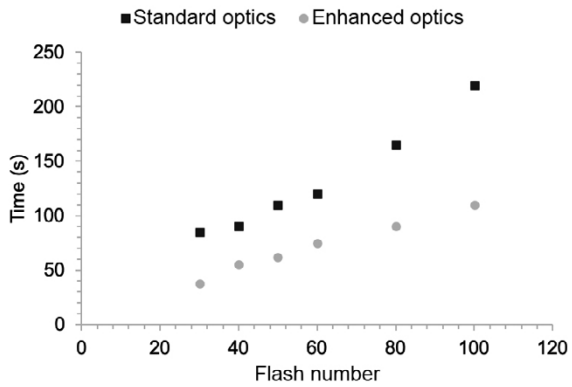

Figure 3: Scatter plot of time versus flash number for measurement of a 96-well plate using the Spark instrument's standard optics (black squares) and enhanced optics (grey circles).

CONCLUSIONS

The data presented here demonstrates the suitability of the Spark multimode microplate reader for IgG quantification using Valitacell's ValitaTiter assay, achieving reproducible and reliable data. It provides an overview of how one parameter – in this case flash number – can significantly affect both the reproducibility between replicates and the overall read times. Selection of the optical system of the reader should therefore match the throughput demands of the lab to allow sufficient read times per plate. The fast and powerful xenon flash lamp of Spark's enhanced optics is ideal for high throughput demands, whereas the standard optics provide a cost-effective solution for labs with lower throughput demands.

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