Simple, rapid & high-throughput IgG quantification in crude cell culture samples using Valita®Titer plates

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Valita®Titer Overview

The accurate, rapid and high-throughput measurement of immunoglobulins, particularly immunoglobulin G (IgG), is essential in the development and subsequent manufacture of therapeutic antibodies.

Here we describe Valita®Titer, a rapid, robust and accurate IgG quantification assay. The Valita®Titer assay range measures IgG concentrations from 2.5 to 2000 mg/L, with a simple add-mix-read protocol. The assay is performed in less than 15-minutes and can be incorporated into any bioprocess workflow in a 96- or 384-well plate format. The assays are high throughput and can be fully automated. Analysis can be carried out in crude cell culture media containing up to 15×10^6 cells/mL with a low sample volume and limited test sample pre-preparation. Assay detection can be performed using fluorescence polarization on a standard microplate reader.

Valita®Titer Assay Principle

Valita®Titer assay quantifies IgG-Fc interactions with a fluorescently labelled derivative of protein G using fluorescence polarisation (FP) for detection. FP effectively analyses changes in the size of molecules (Figure 1).

When you excite a sample with plane polarised light, the probe unbound in solution rotates rapidly leading to depolarization of emitted light. When bound to a higher molecular weight target protein the complex rotates slowly, leading to the retention of polarised light. The amount of target in solution can then be calculated, with the observed output polarisation in a mixture of labeled probe and target being proportional to the fraction of bound probe in solution.
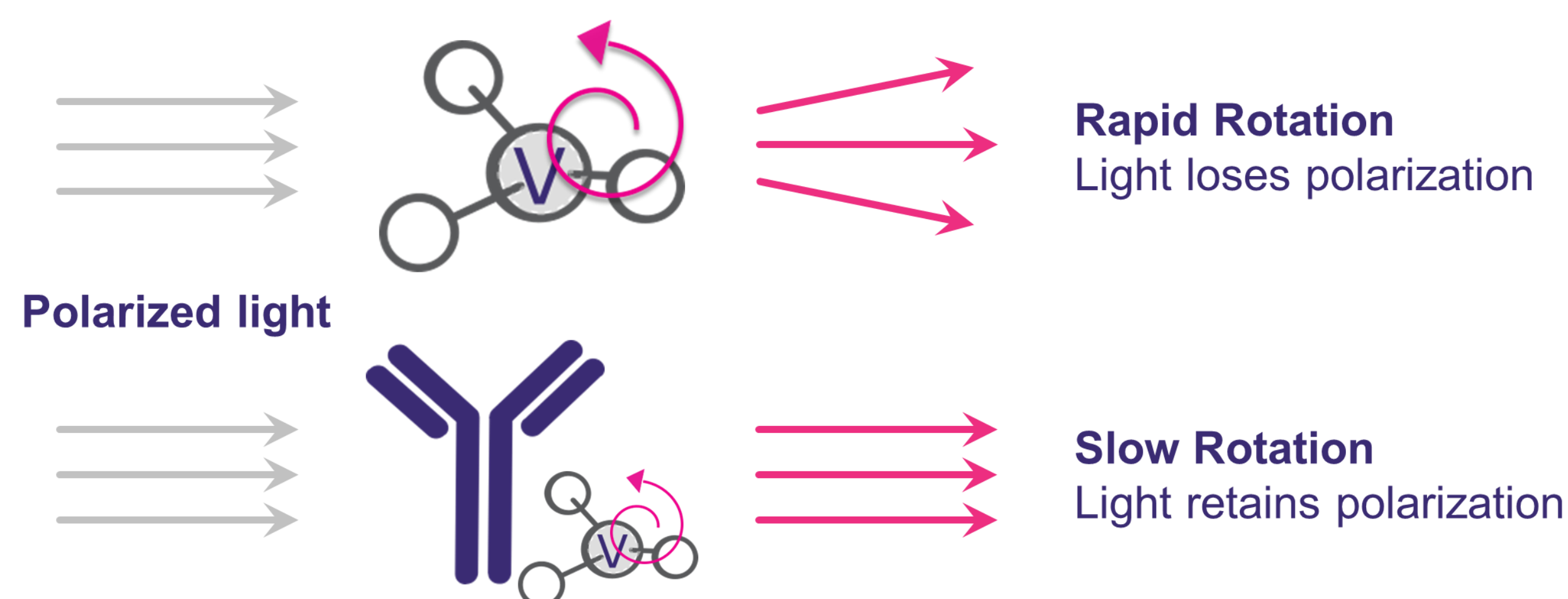

Polarized light

Rapid Rotation Light loses polarization

Slow Rotation Light retains polarization

Figure 1: Small, unbound molecules rotate rapidly in solution (top), while large, bound molecules rotate slowly (bottom).

Valita®Titer Workflow: Add, mix and read

The Valita®Titer workflow consists of 3 simple steps. The plates come pre-coated with an IgG-Fc-specific fluorescently labelled probe. The probe is reconstituted using fresh cell culture media. Following this, IgG standard or test sample is added to the plate. After a short incubation period, the plate is measured using a plate reader with FP. Depending on the plate reader and method, results can be obtained for 96 or 384 crude samples in less than 10-minutes.

Add fresh media & IgG sample and mix

Incubate for 5 minutes at room temperature

Measure IgG using FP on a plate reader

Figure 2: The simplicity, speed & throughput of the Valita®Titer assay make it ideal for both manual & automated workflows.

Valita®Titer Benefits

In addition to the simplicity & speed of Valita®Titer, the assay is robust to cell contamination facilitating crude IgG sample analysis straight in crude samples from cell culture. This eliminates the need for any sample preparation such as filtration or centrifugation to remove cells or purification. Users can simply sample directly from culture to Valita®Titer assay plates & read.
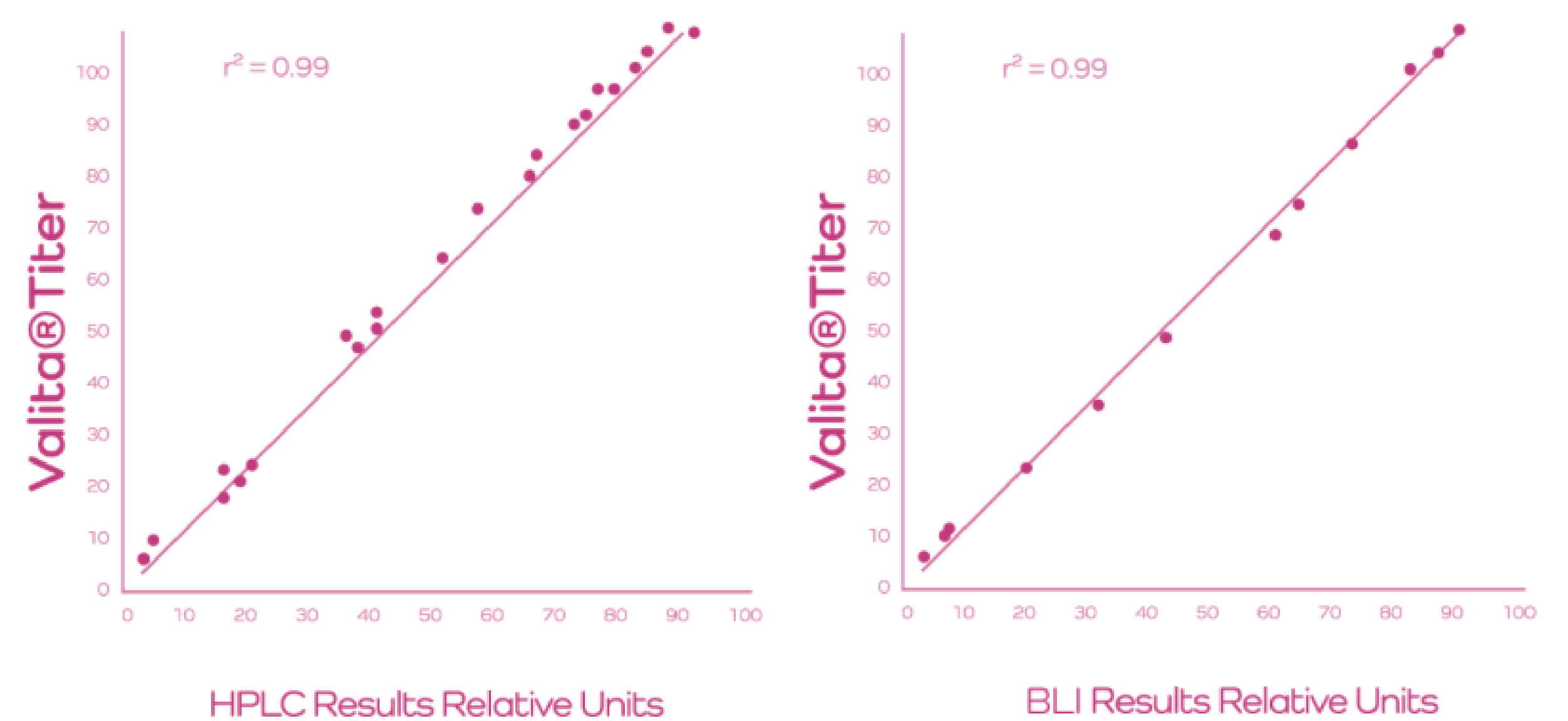Valita®Titer also compares well to industry gold standard techniques for IgG titer measurement.

Figure 4. IgG standard curves quantification. Correlation analysis by Valita®Titer, Protein A HPLC and BLI methods.
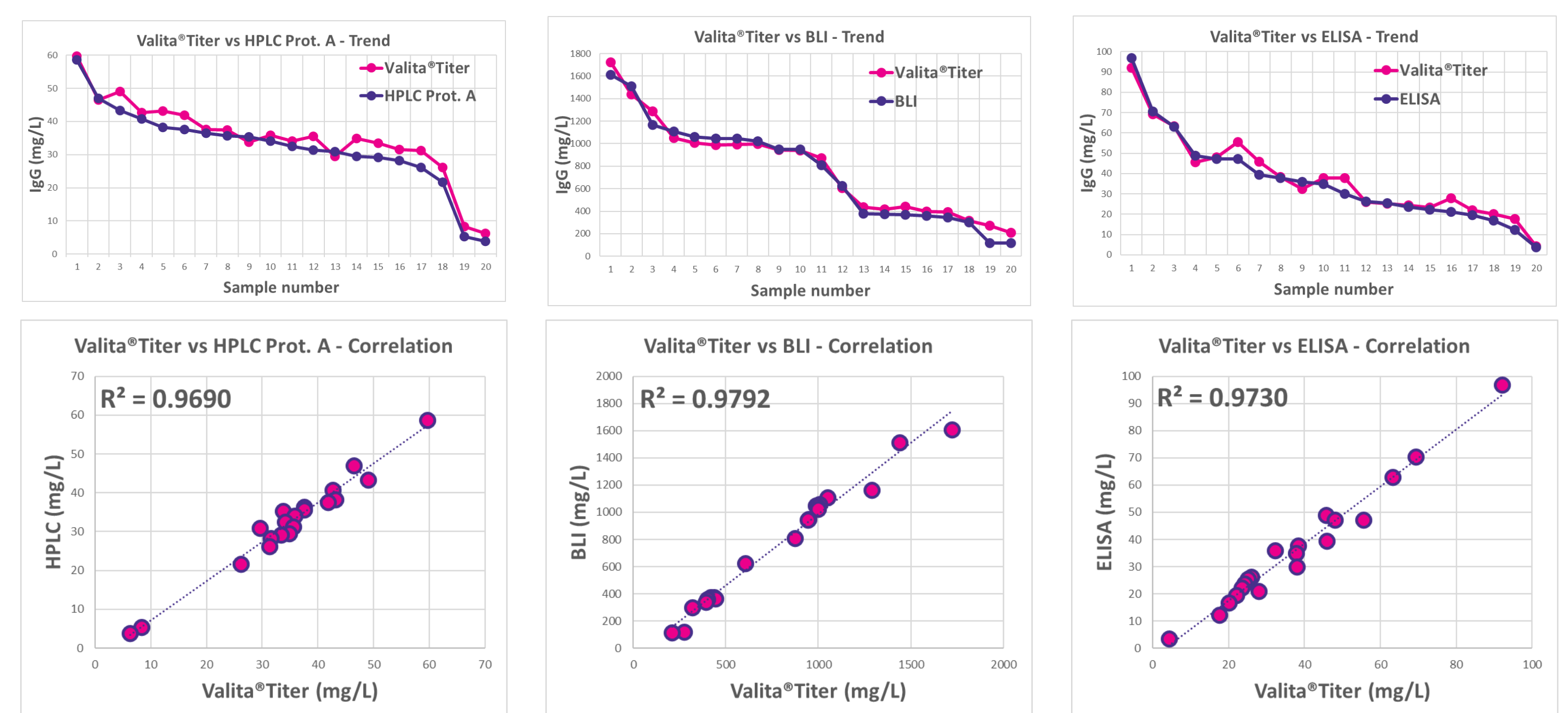

Figure 5. IgG quantification in crude cell culture samples. Trend and correlation analysis by Valita®Titer, Protein A HPLC, BLI and ELISA methods.

In summary, the Valita®Titer assay range has considerable advantages over alternative quantification assays including cost, simplicity, speed and throughput.

	Valita®Titer	BLI	ELISA	HPLC
assay time	10 mins	1 hour	5 hours	10 hours
sample prep	none	none	cell centrifugation	cell centrifugation and protein purification
reagents	1	>2	>6	2
steps	add, mix, read	>3	>20 steps	>5
costs	$	$$$$	$$	$$$

Figure 6: Overview of the key features of the Valita®Titer assay in comparison to industry standard IgG quantification techniques.

Conclusion

- Valita®Titer is a simple, accurate, rapid & automation friendly assay that enables high-throughput quantification of IgGs from crude samples.

- Valita®Titer is robust to cell contamination and facilitates 'straight from cell culture measurement' across a broad functional range.

- The Valita®Titer assay has considerable advantages over alternative quantification assays including cost, simplicity, speed and throughput.