Get high with the Lunatic

Wouldn't it be mind-blowing if you could accurately measure your highly concentrated biologics in a dilution-free, accurate and high-throughput way? The Lunatic platform tackles these issues with confidence; the platform covers a dynamic range from 0.02–200 mg/mL and applies a unique turbidity correction for more accurate quantification.

The Lunatic is your step up to the next-generation UV/Vis reader allowing you to measure up to 96 samples in 5 minutes using only 2 µL of an undiluted sample (Figure 1).

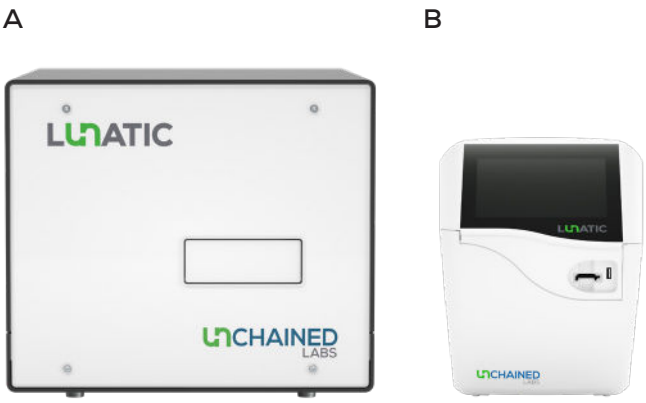


Figure 1: Lunatic: Plate reader analyzing 96 samples in 5 minutes. Fit for manual or robotic sample processing (**A**). Little Lunatic: Chip format reader analyzing 16 samples in 2 minutes (**B**).

Pitfalls quantifying highly concentrated samples

While spectrophotometry allows fast concentration determination of your sample, quantification of highly concentrated samples with high precision and high accuracy is often impeded by:

- **Path length:** the path length needs to be down-scaled (typical 10 mm) and reproducibility is key.
- **Background correction:** such samples tend to form aggregates leading to significant turbidity effects and thus requiring a proper background correction.
- **Detection:** the higher the concentration, the more light is being absorbed by the sample leaving only a marginal amount of light to be detected. As such, an optic system and processing algorithm fit to cope with these amounts of residual light is needed.

Lunatic's solutions

Lunatic offers the unique possibility to measure samples from 0.03–275 OD. Precision at both ends of this series is guaranteed using two types of Lunatic Chips and Lunatic Plates with fixed path lengths. The Lunatic Chip and Lunatic Plate have a path length of 0.5 mm allowing measurements up to 40 OD. The High Lunatic Chip and High Lunatic Plate, however, have two micro-cuvettes (0.1 and 0.7 mm) extending the dynamic range to 275 OD.

Quantification of turbid, highly concentrated samples can be troublesome and requires top notch turbidity correction. Conventional UV/Vis instruments use one wavelength at which to perform a background correction, such as 340 or 405 nm, where no molecule-specific absorbance is expected. This holds true if the scatter of the light at each wavelength is the same, which is not the case (Figure 2). Therefore, on the Lunatic, a turbidity profile taking wavelength-dependent scatter profiles into account was created, allowing an optimal background correction. Nevertheless, an application performing a single point background correction at 340 nm is also available on the Lunatic. In case of pure samples, both the Protein (Turbidity) and Protein (Single point) Classic A280 apps will return very similar results. More turbid samples however require the sophisticated turbidity correction of the Protein (Turbidity) application for accurate concentration determinations.

Being able to measure low and high protein concentrations sounds great but it doesn't tell you anything about the precision and accuracy. To investigate this, we made a comparative study quantifying a gravimetric IgG dilution series ranging from 0.02 mg/mL

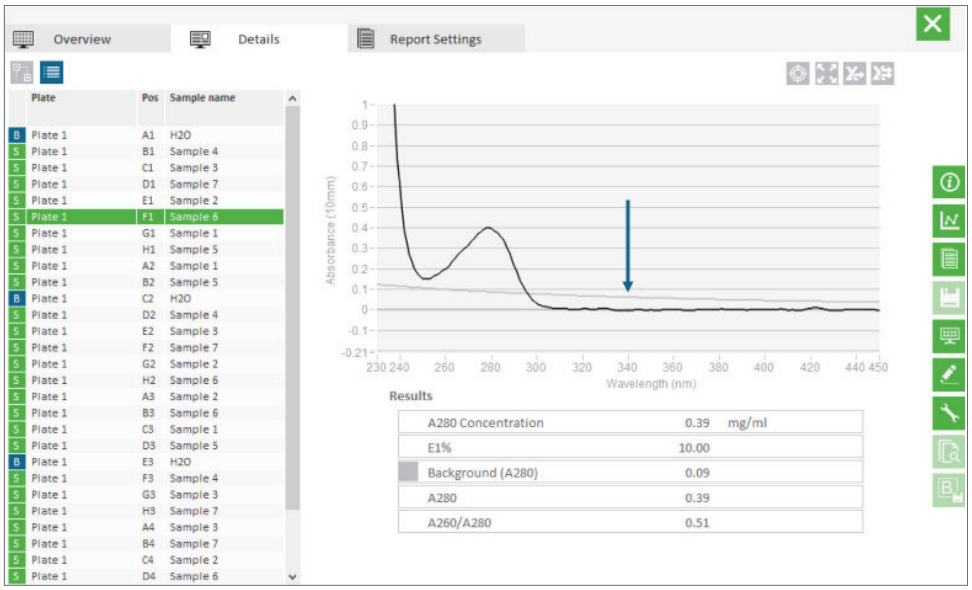


Figure 2: Turbidity correction. The Lunatic platform performs a turbidity correction that accounts for wavelength-dependent scatter profiles (gray line on the graph) rather than choosing the absorbance at one specific wavelength, like 340 nm, to correct the entire UV/Vis spectrum.

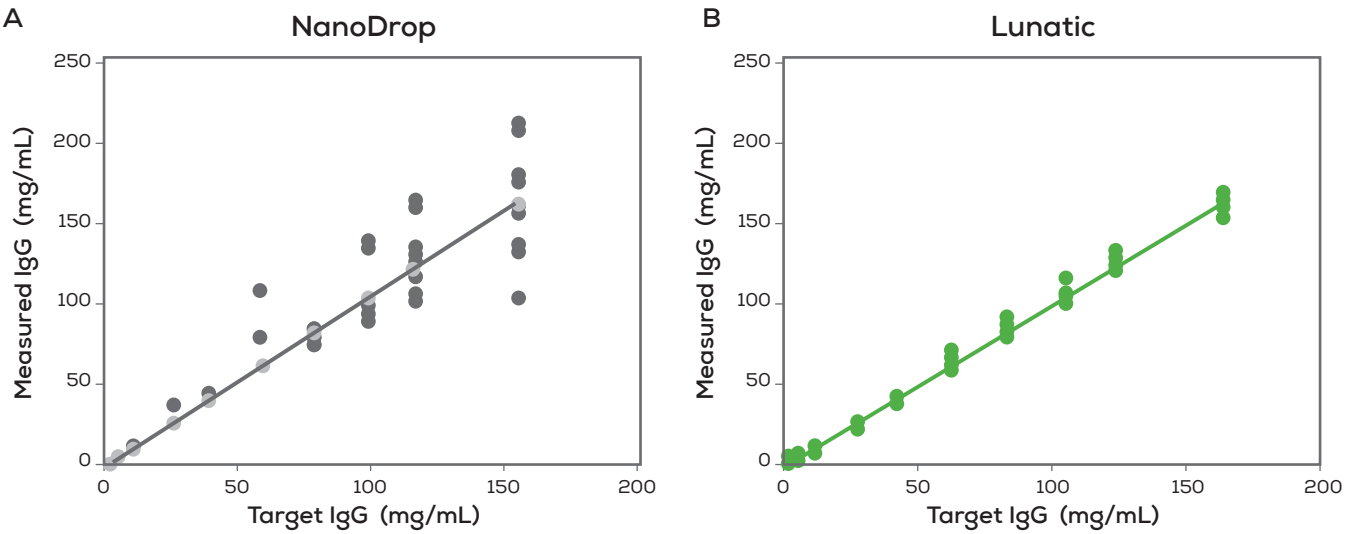


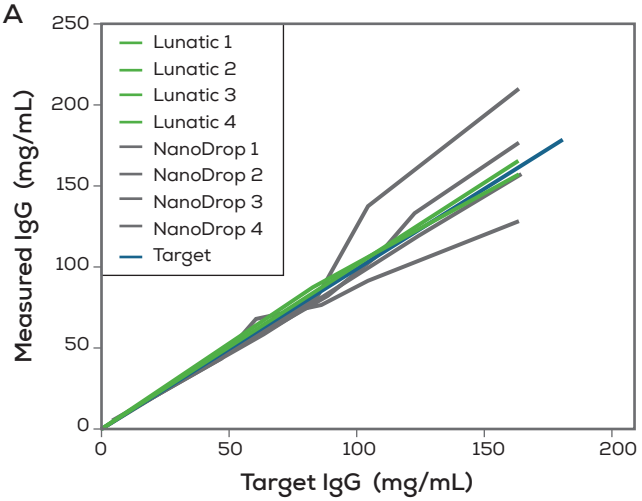
Figure 3: Results for a gravimetric IgG dilution series on four NanoDrop (**A**) and four Lunatic (**B**) instruments.

up to 160 mg/mL on two of the larger Lunatic systems, two Little Lunatics, three NanoDrop2000 and a NanoDrop One UV/Vis spectrophotometer.

At the start, each instrument was subjected to a verification test with an aqueous potassium dichromate ($K_2Cr_2O_7$) solution as recommended by the manufacturers. The OD value at 350 nm was monitored, allowing an OD value of 0.657 ± 0.011. All instruments complied with this specification. Subsequently the gravimetric IgG dilution series was measured in octuplicate on Lunatic and on all Nanodrop instruments. On the little Lunatic quintuplicate measurements were performed. The mAb was formulated in 5 mM NaSuccinate and 60 mM Trehalose at pH 5.0.

Individual measurements on all instruments assessed are displayed in Figure 3. The standard deviation on NanoDrop instruments ranges from 0.01 mg/mL for low concentrated samples up to 29.81 mg/mL on the highest concentrations measured. On Lunatic instruments, standard deviations between 0.01 and 4.10 mg/mL are observed going from low to high concentration measurements.

A



B

IgG (mg/mL)	Lunatic	NanoDrop
0.1	3.70%	7.00%
0.4	1.42%	9.57%
1.0	0.38%	2.95%
4.0	0.37%	1.07%
10.0	0.20%	3.12%
25.3	0.14%	3.49%
40.4	0.42%	3.85%
60.9	1.30%	7.35%
81.2	1.68%	3.59%
103.3	1.22%	19.06%
121.8	0.60%	19.02%
162.2	2.69%	20.31%

Figure 4: Comparison of the average concentrations per instrument. NanoDrop and Lunatic instruments are represented by gray and green lines respectively, target concentration is annotated by a blue curve (**A**). The percentage of variation among instruments (**B**).

In order to investigate if the standard deviations observed are instrument-specific or rather random, we compared average absorbances for all instruments (Figure 4). Standard deviations measuring a 0.1 mg/mL sample on the Lunatic instruments vary from 0.005–0.009 mg/mL. Identical measurements on the NanoDrop instruments result in a similar standard deviation range (0.003–0.011 mg/mL) indicating that replicate measurements on NanoDrop instruments are as reproducible whilst correlation between different instruments is low. Table 1 puts the inter-instrument variation observed in Figure 3 into numbers. In all concentrations assessed, Lunatic results correlate better than NanoDrop results do. Especially in the lower and higher end of the dilution series tested, large inter-instrument variations on the NanoDrop instruments can be observed. For example, analysis of a 162.2 mg/mL sample on multiple Lunatic instruments results in 2.69% variation while 20.31% variation on multiple NanoDrop instruments could be observed.

In addition to the percentage of variation among instruments, we also assessed the percentage of bias (calculated as ([measured]–[target]/[target]). The percentage of bias looks at how closely concentrations approached the target concentration. Similar to the inter-instrument variation analysis, large divergences between the NanoDrop instruments (% bias ranges from -20.4% up to 33.5%) can be observed whereas Lunatic instruments all closely approach the target concentration (% bias ranges from -2.4% up to 7%).

Conclusion

Lunatic allows quantification of low to high concentrated samples in a fast, high-throughput way with high precision and high accuracy. Although NanoDrop measures samples with similar precision, large inter-instrument variations could be observed resulting in an overall low accuracy. Lunatic is your instrument of choice as it will return the same results for the same sample over and over again, independent of instrument used.

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Rev B