Qualifying and Quantifying Plasma Samples with Lunatic

Introduction

Plasma-based research and biobanking programs have generated abundant interest in cell-free nucleic acid analysis and biomarker discovery. Although the quality of plasma can severely impact the success rate of these processes, plasma is typically scored by its perceived color and clarity. Visual assessment of plasma samples can bias their scoring since plasma samples can have the same visual appearance (i.e. color and clarity) yet contain different biological components. Biologically, these parameters are related to hemolysis (pink-red color), icteric serum (orange-dark yellow color), and turbidity (transparent to opaque). Lunatic software can distinguish these samples by quantifying the hemoglobin, bilirubin and protein content, in addition to sample turbidity. These parameters can be used to score plasma quality objectively and be correlated to upstream extraction procedures or downstream performance, which is beneficial for all studies requiring plasma and serum.

Lunatic makes batch quantification of biologics and nucleic acids a no-brainer. Combining high speed UV/Vis spectral analysis with micro-volume Lunatic Plates (Figure 1), Big Lunatic offers the unique possibility of quantifying 96 droplets at once in only 5 minutes. Lunatic Plates use just 2 µL of sample and there's no need to worry about sample dilutions, contamination, or evaporation (Figure 2). The Lunatic Plate takes care of your samples upon loading them by hand or using a liquid robot. Just drop, load and read.

This application note guides a user through the Plasma QC application on Big Lunatic. This application is specifically designed to assess the quality of plasma samples by identifying the plasma-relevant components contributing to the total UV/Vis absorption spectrum. As with other Unmix applications, accurate quantification of the molecule of interest is obtained from its isolated spectrum using a deconvolution algorithm built into Lunatic software. This application is also available on Little Lunatic instruments, which can run up to 16 samples at a time in just 2 minutes using Lunatic Chips.

Figure 1: Big Lunatic can analyze 96 samples in 5 minutes and is fit for manual or robotic sample processing.

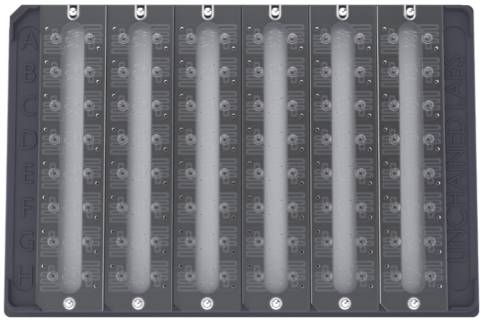


Figure 2: Lunatic Plates have 96 individual microfluidic circuits that enable reads on just 2 µL sample.

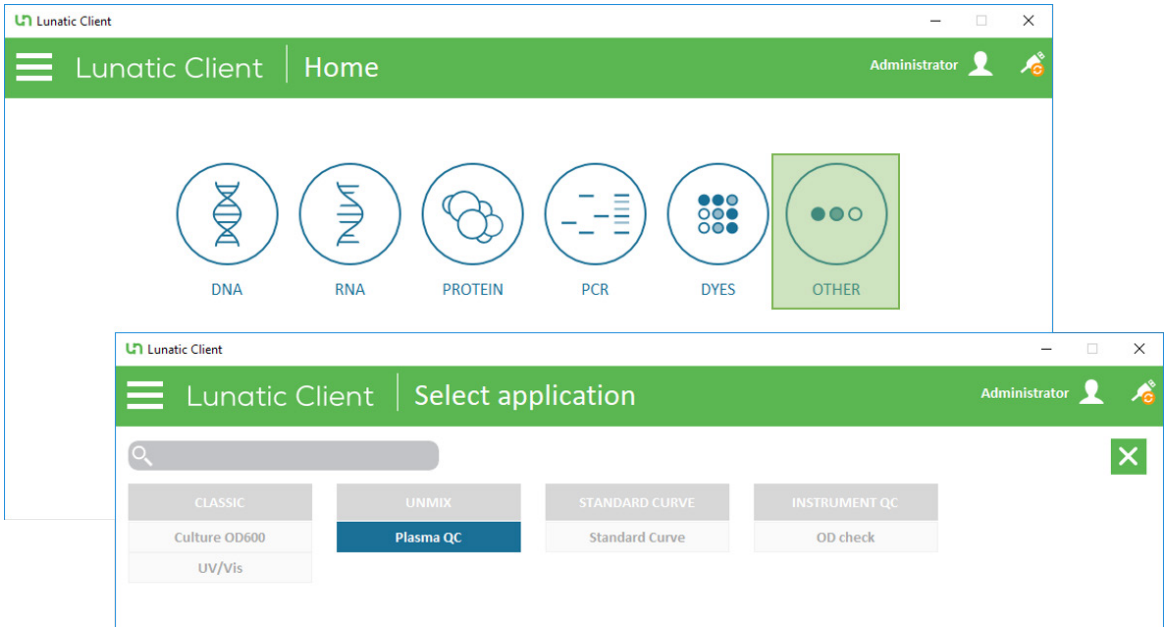


Figure 3: Selection of the Plasma QC application in Big Lunatic Client software.

App selection

On Big Lunatic, the Plasma QC application can be found in the "Unmix" column upon selection of "Other" in the Sample Type screen (Figure 3). Like other Unmix applications, ensure that pure water is used as a blank. Aside from sample names, no additional user input is required. The High Lunatic Plate, equipped with two micro-cuvettes with pathlengths of 0.1 and 0.7 mm, is loaded with 2 µL of each sample and read on Big Lunatic.

Results

Spectral Visualization

Plasma samples typically contain a high concentration of protein, ranging from 60-80 mg/mL, giving rise to large absorption peaks at 280 nm. As hemoglobin and bilirubin absorption peaks tend to be much smaller, these absorption events are easily masked in the typical visualization of a UV/Vis spectrum from 230-750 nm. Therefore, while the total protein concentration is calculated and reported, for proper visualization of the Unmix spectral fitting, Lunatic Analysis software displays the absorption spectrum from 350 to 650 nm (Figure 4).

The Plasma QC application measures the UV/Vis spectrum of the sample and isolates these specific components:

- **Hemoglobin (shown in red)**: The concentration is calculated using the A410 peak value of this profile multiplied by the concentration factor of hemoglobin (13.23).
- **Bilirubin (shown in orange)**: The concentration is calculated using the A450 peak value of this profile multiplied by the concentration factor of bilirubin (1.24875).
- **Protein (shown in green)**: The concentration is calculated using the A280 peak value of this profile using a default E1% extinction coefficient of 10 for complex protein mixtures.
- **Turbidity (shown in gray)**: The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum (black curve).

The Residue value (RRSE) is the percentage of the measured spectrum which could not be annotated, representing the quality of fit. This parameter is displayed as a yellow curve as well as a percentage value below the graph. A warning sign will appear for samples with a residue value above 2.5%, which is typically due to (1) high sample turbidity, (2) presence of an unknown component, or (3) low-concentration samples.

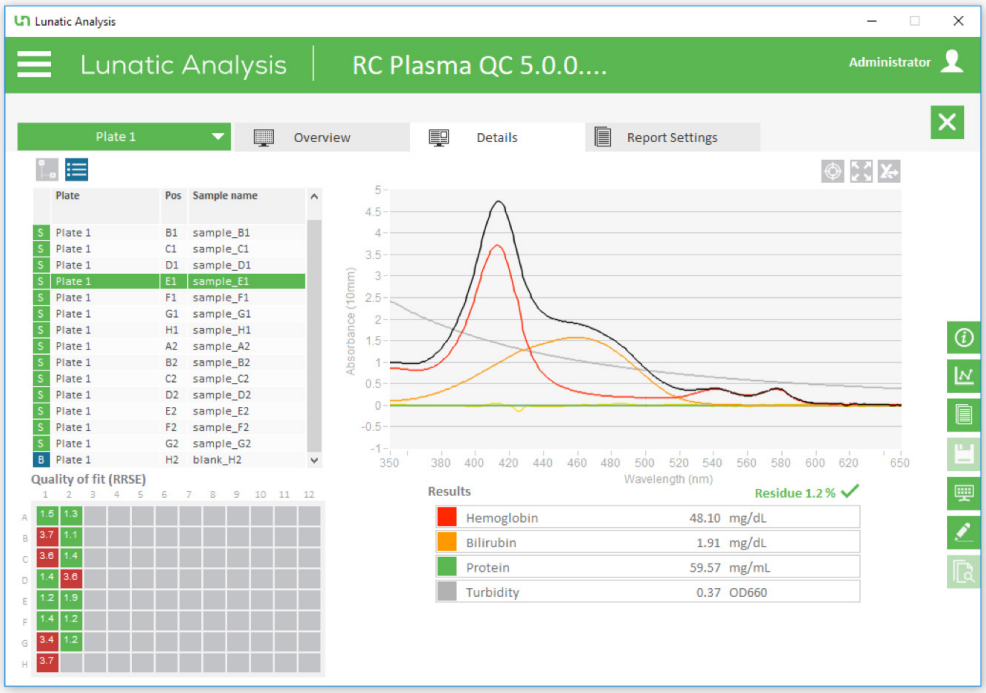


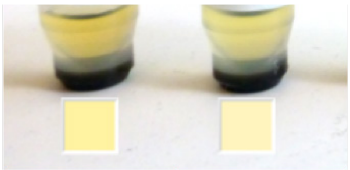
Figure 4: Big Lunatic Analysis software quantifies the plasma-relevant components from the measured UV/Vis absorption spectra.

Reports

A variety of report types are generated for exporting and visualizing the data: HTML, XML, TXT, XLSX, PDF and CSV files. In addition, the Big Lunatic platform enables customization of reports with a flexible selection of content.

Objective scoring of plasma samples

Individual scoring of plasma samples by color and clarity remains imprecise, labor-intensive and operator-subjective. Lunatic can differentiate plasma samples with similar visual appearance by quantifying the biologically-relevant components: hemoglobin caused by hemolysis, bilirubin due to icteric serum, total protein, and turbidity caused by high lipid content. In the plasma samples shown in Figure 5, an approximate 7-fold difference in bilirubin content was measured, which correlated to a downstream 18 % difference in cell-free DNA yield. By using data-driven binning of quality prior to downstream analysis, researchers can determine plasma sample threshold values appropriate for their workflow.



Color, clarity	Dark Yellow, transparent	Yellow, transparent
Hemoglobin (mg/dL)	33.14	10.63
Bilirubin (mg/dL)	**3.06**	**0.43**
Protein (mg/mL)	67.83	60.87
Turbidity (OD660)	0.18	0.07

Figure 5: Lunatic quantifies the biological components of plasma samples with similar color and clarity.

Conclusion

Tired of squinting to check for plasma sample quality, and then getting subjective values that differ between researchers? With only 2 µL of sample, Lunatic can do quantitative spot checks for you by measuring the full UV/Vis spectrum and independently quantifying the plasma-relevant components: hemoglobin, bilirubin, and protein, along with sample turbidity profiles. This information provides researchers with operator-independent, objective scoring of plasma

samples, which can be used to assess upstream collection procedures or downstream performance. Lunatic enables high-throughput analysis and quantification of plasma, so you can take the guess-work out of plasma quality determinations.

Acknowledgements

We acknowledge RUCDR Infinite Biologics and Brooks Life Sciences.

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

Rev A