Quantification of unknown samples using standard curves

Introduction

A standard curve represents the relationship between absorbance and known sample concentrations and is used to determine unknown sample concentrations. The Standard curve application can be used to quantify unknown sample concentrations by measuring a new standard curve or using a previously saved one. Depending on the quantification assay being used, it is possible to use the absorbance at 1 particular wavelength or to use the difference in absorbance between 2 wavelengths (defined by the user). Using the 2 wavelength method leads to increased precision (see below).

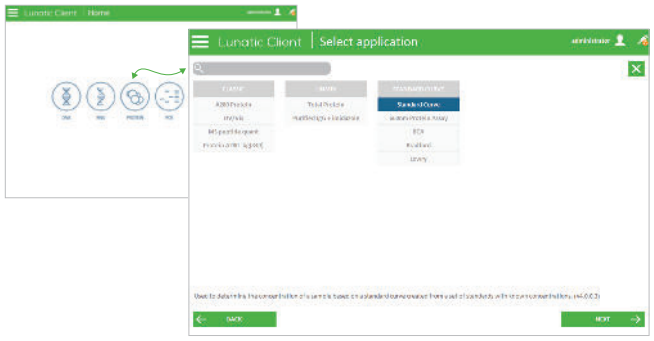

Figure 1: Illustration of the Lunatic interface. The image in the back shows the Sample Type screen, whereas the image in the front displays the available applications for the selected Sample Type.

App selection

On Lunatic, the Standard curve application can be found in the "Standard curve" column present with each Sample type (Figure 1). On Little Lunatic, this application can be found on the applications screen (Figure 2). Background correction is calculated using a region in the measured spectrum which has the lowest absorbance which means this application can be run without measuring a blank solution. Aside from sample names, additional user input will be requested:

New standard curve: define reference values and standard curve type, units and wavelength(s) of interest.

Saved standard curve: choose saved standard curve and standard curve type.

Figure 2: App button on the Little Lunatic app selection screen.

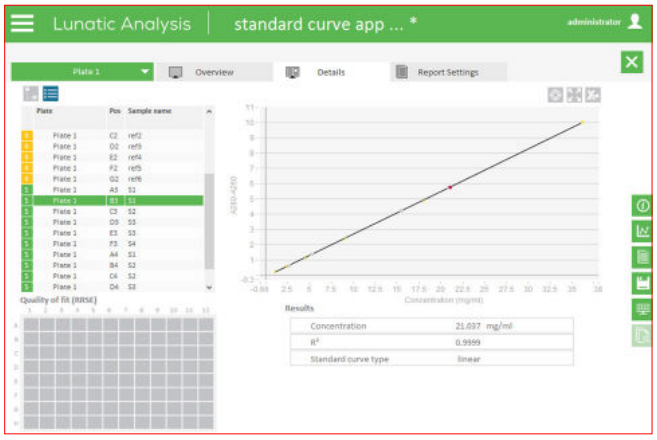

Figure 3: Illustration of the Results screen on the Lunatic. Measured standards (shown as yellow crosses) are used to fit a standard curve. This standard curve is then used to calculate unknown sample concentrations (gray squares, red if selected).

Results on screen

Measured standards (shown as yellow crosses, Figures 3 and 4) are used to fit a standard curve. This standard curve is then used to calculate unknown sample concentrations (gray squares, red if selected).

Concentration: calculated by the regression based on the standard curve derived from the selected references.

R^2: the coefficient of determination, a measure of how good the references are correlated to the fitted regression line.

Standard curve type: regression curves and respective formulas defined in Table 1.

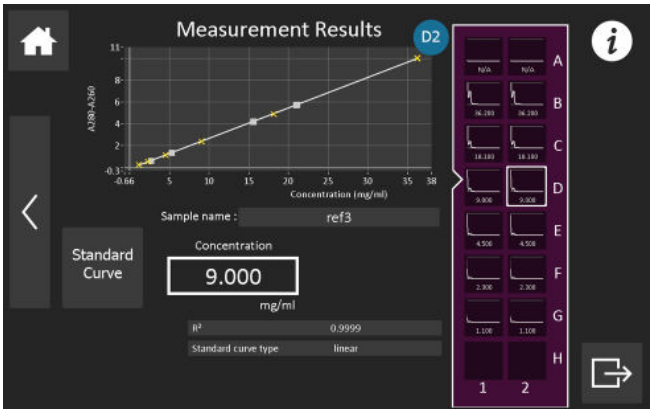

Figure 4: Illustration of the Results screen on the Little Lunatic. Measured standards (shown as yellow crosses) are used to fit a standard curve. This standard curve is then used to calculate unknown sample concentrations (gray squares, red if selected).

Report

A variety of report types are generated: an HTML, XML, TXT and a CSV file are created on both systems. In addition, Lunatic also creates XLSX and PDF report files. On the Little Lunatic fixed report templates are used while the larger sytem allows full flexible selection of the content to be reported.

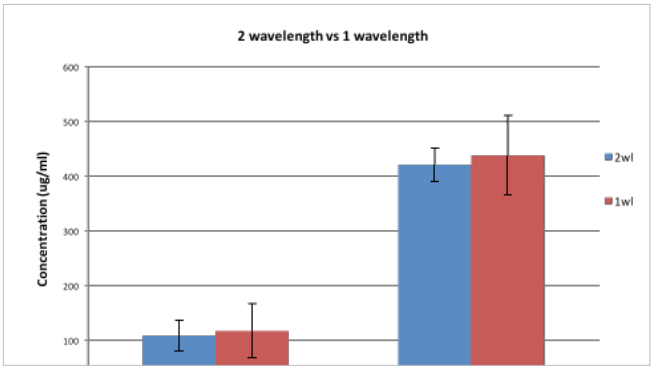

Figure 5: Protein quantification using 2 wavelengths vs 1 wavelength.

Additional information

The 2 wavelength method leads to increased precision as there are smaller standard deviations (Figure 5). Table 1 on the other hand highlights the different types of standard curves.

Type	Formula	Remarks/use
Proportional	$y = a^*x$	Used in the same way as linear, but fitted through zero
Linear	$y = a^*x + b$	Line used as best fit between all data points, when values appear to lie on or scattered around a straight line
Point-to-point	-	Linear connection between consecutive points
Quadratic	$y = a^*x^2 + b^*x + c$	When overall shape of the data points seem to curve, a parabola is fitted
Quadratic (through zero)	$y = a^*x^2 + b^*x$	Same as normal quadratic but fitted through zero
4 parameter curve fit	$y = \left[\frac{a-d}{1+\left\{\frac{x}{c}\right\}^b}\right] + d$	Used when standard curve contains asymptotes (e.g a sigmoidal shape)

Table 1: Different types of standard curves and their respective formulas. (x= concentration, y= absorbance).

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 B