ThrombinCL

Notes for Analysing Thrombin Generation Curves

A help tab in the app summarises the main features of ThrombinCL version 0.9 and above. Detailed help is presented below.

Calibrator tab

Loading data

The app opens with the Calibrator tab and example files of calibrator and sample data are loaded. The 'browse' buttons are used to find and load your own data. Excel (xlsx), csv or txt files are accepted and the file type does not have to be specified. If Excel files have more than one sheet, you can specify the sheets containing the calibrator and/or sample data. All time course data should be formated as a single column of time in column 1 followed by columns of fluorescence data and all columns are assumed to have headings in the first cell.

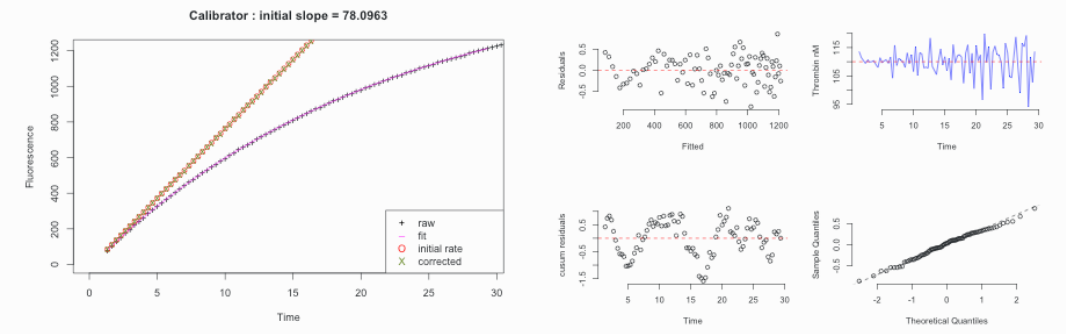
Selection boxes allow the user to specify a range of columns ('start col', 'end col') to use as calibrator or sample data. A drop down menu is provided to select which calibrator curve is being analysed if more than one is available.

Calibrator fitting

The first task is to fit a curve to the calibrator and correct for the loss of signal due to substrate depletion and inner filter effects. The figure below shows (left graph) raw data (+, black) and fitted line (-, magenta), the calculated initial rate of the response (O, red) and the fitted response corrected for loss of signal (X, olive). The panels on the right interrogate the fitting further. The residual plots should be as random as possible; the quintile plot should be linear; and the thrombin plot should be as consistent and noiseless as possible. The thrombin response will become noisy over time but this is a natural consequence of the fitting algorithm and first derivative transformation.

One of the selection boxes on the left of the figures allows for the number of points to be specified for fitting. Generally speaking, fewer points improves the fitting, but enough points should be included to cover the change in fluorescence observed in the samples over the time period where interesting data are collected and analysed.

When calibrator curves have been fitted satisfactorily, the slope obtained (or an average value from several curves) in entered into the 'Calibrator initial rate' box on the left. Calibrator concentration is entered into the 'Conc of calibrator' box, and if all is well this should match the red line on the Thrombin concentration plot (top right panel below)

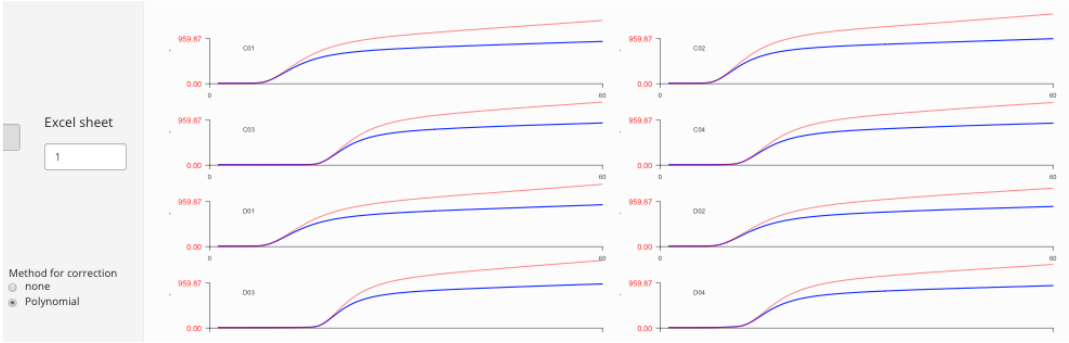


Sample data

Once the sample data are loaded, the raw fluorescence curves are shown on the bottom right hand side of the 'Calibrator' page (example shown below).

Data are selected using the 'start col' and 'end col' boxes to match up with the calibrator selected above. The display is organised using the 'plot nrow' box. It is possible to exclude unwanted late data points in the selected sample data using the 'truncate data' box.

The graphs on the bottom right of this page (example below) show raw fluorescence or split the curves into raw fluorescence and fluorescence corrected for signal loss. Selection is made using the radio buttons labelled 'none' and 'polynomial' under 'Method for correction', as shown below. The difference between uncorrected (blue line) and corrected (red line) fluorescence is clearly visible over time.



Plots and curve tabs

The plots and curve tabs show transformed data, that is the first derivative plots of fluorescence data, with or without polynomial correction, as selected.

At the bottom of the left panel, under 'Transformed curves' there are options for what is displayed and analysed. By default, 'lag % for start' is set to 10% meaning a change of 10% in the transformed response signals that the lag period is over. 'Smooth tail' is the number of points selected at the end of the transformed response that gives a stable signal for thrombin-alpha-2-macroglobulin complex only and no more free thrombin is being generated.

The radio buttons under 'Display' determine what curves will be displayed on the 'plots' and 'curves' tabs and what results will be generated:

F = fluorescence

Thrombin = Thrombin concentration calculated from the calibrator concentration and rate entered earlier

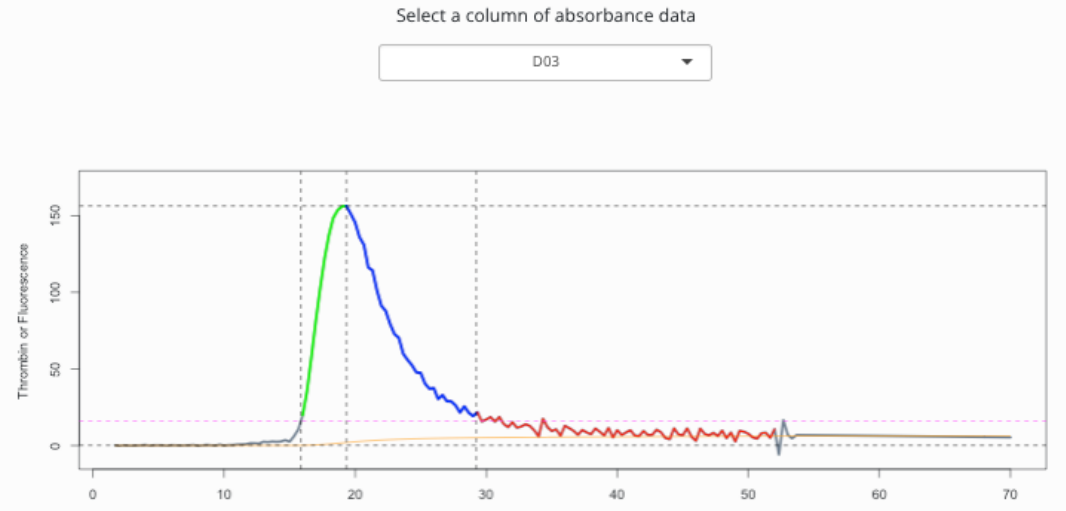
Smooth= shows the region of curve smoothing

no T-alpha2M = subtracts the contribution of the thrombin-alpha-2-macroglubulin complex.

An example thrombin response showing smoothed tale taken from the 'Curve' tab is shown below. The green line begins after the lag phase and ends at the peak where the blue line continues to the beginning of the tail, shown in red. The gold line is the formation of alpha-2-macroglobulin complex.

Dotted lines indicate time to end of lag phase, time to peak height, peak height and time to tail. All these results are provided in a table underneath the selected curve.

The 'Plots' tab shows all the curves and a selected parameter.



Results

The 'Results' tab collects all the results from all the curves in a table format and can be copied and pasted for further analysis. All the parameters shown can be observed on the thrombin generation curves in the 'Plots' and 'Curve' tab, with the addition of 'Base' which is the time between end of lag and tail and 'LagtoPeak' which is time from the end of the lag phase to the peak thrombin, as a measure of how fast the initial burst of thrombin generation is. The area under the curve (AUC) is calculated as the thrombin generated between the lag phase and the start of the tail, i.e. including the area bounded by the green and blue lines and the 'Base'.

Table of all results

Sample	Ao	Lag	AUC	Peak	ttPeak	ttTail	LagReading	Base	LagtoPeak
C01	-0.13	7.34	978.06	113.05	11.67	24.18	11.19	16.84	4.32
C02	-0.59	7.08	1039.22	135.09	10.67	22.00	12.98	14.92	3.58
C03	-0.25	15.84	1008.11	134.84	20.00	30.54	13.26	14.70	4.16
C04	0.04	11.00	991.20	122.82	15.00	27.10	12.32	16.11	4.00
D01	-0.30	7.52	954.33	103.27	12.33	25.24	10.06	17.72	4.81
D02	-0.04	7.82	935.24	97.48	11.67	26.39	9.71	18.57	3.84
D03	0.17	15.85	1038.88	156.37	19.33	29.13	15.79	13.28	3.48
D04	-0.51	11.64	1007.55	131.37	15.33	26.56	12.68	14.92	3.69

Settings

The settings tab provides a table with applied settings used in the analysis as an aid to record keeping and reproducible analysis. The table can be copied and pasted into other applications.

Help

The help tab is a summary of this document for handy reference. It also contains a dialog box and drop down menu to change the look of the app if alternative colours or fonts are preferred.

Useful links

R code, data and help files are available from: https://github.com/drclongstaff/Thrombin_Generation_Assays/

Summary of other apps available at: https://drclongstaff.github.io/shiny-clots/

More information and links may be found at http://www.nibsc.org/science_and_research/biotherapeutics/haemostasis/fibrinolysis.aspx