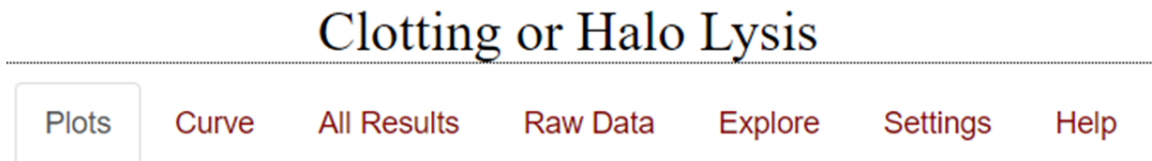


Clot_or_HaloCL

Notes for analysing clotting fibrinogen or plasma or fibrinolysis by the halo method

The main window opens with a series of Tabs to help the user perform different tasks. The *Help* tab in the app summarises the main features of Clot_or_HaloCL version 0.2 and above. More details are presented below for each Tab.



The *Plots* tab

Data Entry

The app opens with a set of supplied data, which can be used to get a feel of the options. The next step is to load your own data for plotting and analysis using *select dataset* and *Browse*

Select dataset:

File type

csv
 csv2
 txt
 xlsx

Arrange data and clot/lysis point

How many rows?

% change (0.5 is 50%)

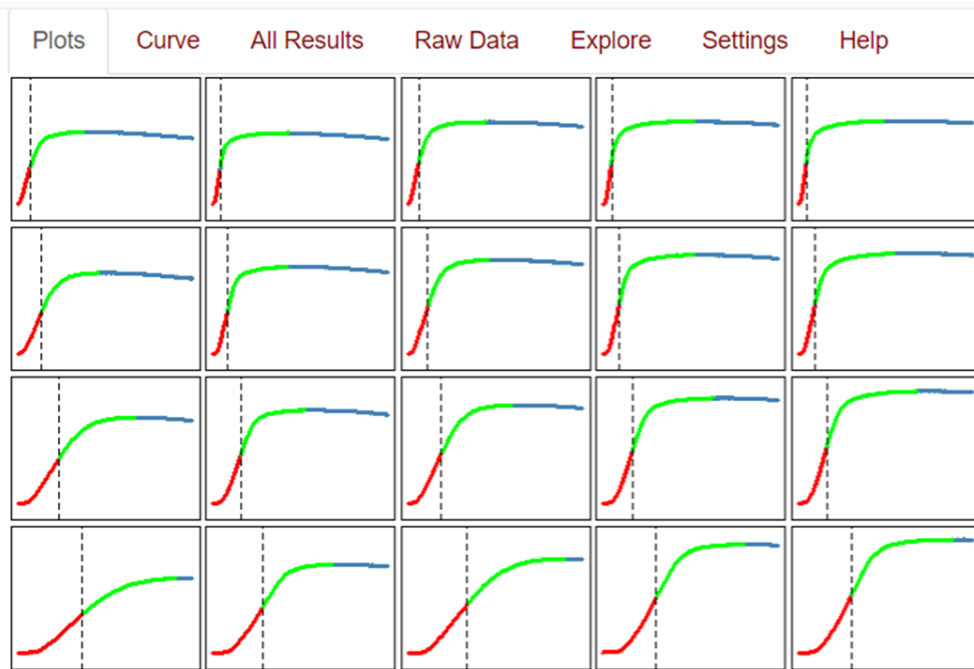
Time course data should be formatted as a single column of time in column 1 followed by columns of absorbance data. The program detects the length and width of the data so there is no need to specify these dimensions. Data can be read as csv, csv2 (using , instead of . for the decimal point), txt or Excel files. These options are specified using the radio buttons in the left hand panel. It is also necessary to specify if the columns have header text (“Time”, well names “A1”, “A2”... etc), which is recommended.

Note: *it is important not to leave empty cells, incomplete columns or rows, or spaces in names of column headers in any data files. Gaps and spaces are the main reasons the program fails to read data or complete an analysis.*

Below is an example of a few rows of data to show how it should be formatted (and how it should appear in the *Raw data* tab).

Time	A2	A3	A4	A5	A6	A7	B2
0	0.05	0.04	0.041	0.04	0.04	0.041	0.041
30	0.051	0.041	0.041	0.041	0.041	0.042	0.042
60	0.052	0.042	0.042	0.042	0.042	0.043	0.042
90	0.054	0.044	0.044	0.043	0.044	0.044	0.042
120	0.057	0.048	0.045	0.045	0.046	0.045	0.043
150	0.062	0.053	0.049	0.05	0.051	0.047	0.042
180	0.069	0.06	0.058	0.06	0.059	0.052	0.043
210	0.078	0.068	0.07	0.073	0.066	0.058	0.043

Plotting the data



The graphical output in the main panel of the opening page is organised by number of rows specified, using the *How many rows* numerical input box. Curves in the panels are modified by *% Change* input and the sliders that control the *x and y axis ranges*. It should be noted that the axis options do not influence the way the data are analysed, only their graphical display. The *% Change* setting obviously affects the results of the analysis.

The supplied data give an output as shown below for 50% clot formation (also corresponding to 50% clot lysis in the halo fibrinolysis method). Example plots are shown below. Sections of curve are colour coded to designate the points to selected change and maximum.

Fitted data and fitting options

The next series of options affect the precision of the results.

Answer at nearest point or interpolate between points

Use raw data or fit additional points

Settings for fitting additional points

fitting start truncate time by n points

0 0 1000

Adjust graphs

x axis range

0 3,580 22,500

0 2,250 4,500 6,750 9,000 11,250 13,500 15,750 18,000 20,250 22,500

y axis range

0 0.5 5

-0.1 0.45 1 1.55 2.1 2.65 3.2 3.75 4.3 4.85 5

Find points

Nearest point

Interpolate

Data source

Raw data

Fitted points

Nearest point will find the closest point in the raw data for your analysis; *Interpolate* is more precise as it calculates a fitted value between the data points supplied. *Nearest point* is more robust and will work better with irregular curves or flat or noisy data if interpolate fails. The next pair of radio buttons allow you to use the raw data or generate additional points to a spline curve, which is useful for improving precision if you have sparse data. You can narrow the region of interest for fitted data points using the set of input boxes to specify *fitting start*, *truncate time by* and *n points* (number of points). The newly generated data are shown in the graphical output sections on this page and the *Curve* tab, and also in the *Raw data* tab. The default options in this section are *Interpolate* and *Raw data*, which should work well for regular curves.

Baseline options

There are several ways to set the baseline.

Baseline options

global zero global zero nth point
 nth absorbance
 min abs + offset below

The baseline options are set using the radio buttons with numeric inputs from the adjacent boxes. The zero value selected will be the absorbance that equates the starting value before clotting (or lysis in the halo method). The first option is for a global zero, which is specified the numerical input box below *global zero*, and may be the starting absorbance for example, if this is consistent. In this case all curves will have the same zero absorbance value.

A second option provided is to set zero for each curves at a particular time point. The default value for the *nth absorbance* is 1, i.e. the first absorbance reading of each curve. Later points can be chosen by increasing the value in the *nth point* input box. With this option, individual curves may have different zero absorbance values but they will all be selected at the same time point.

The third option is to use the minimum absorbance value of each curve with the possibility to add an offset value using the slider below the radio buttons. This is useful if there is drift or noise and some manual adjustment is required. In this case the zero absorbance can vary for each curve and will likely come from a different time point along the curve.

The way zero has been set is recorded in the table in the *Settings* tab. Some care is needed when using these options as the chosen zero affects several of the calculated results. The Results Table can display the absolute maximum absorbance or the absorbance above the baseline after subtraction of the chosen zero value.

Maximum absorbance options

There are two ways to set the maximum absorbance values for the curves.

Maximum absorbance options

global max

each curve max

global max

1

global max sets a value that applies to all the curves and the value is provided in the numerical input box. This may be useful if you expect a particular maximum value but your curves have not all reached the maximum during the run. This value is also useful for halo assays analysis that include some non-clotted sample wells. Alternatively, *each curve max* identifies the maximum absorbance for each curve and sets this to a maximum. In this case all the curves may have different maxima.

Results table

The results table (also shown in the figure above), corresponds to the graphical layout and displays the results selected using the radio buttons in the left panel, summarised below.

- **Column names:** Displays the header text in the data file in the specified arrangement
- **Chosen zero:** Displays whatever zero value has been selected for each curve
- **Time to % change:** Time to chosen % clotting or lysis from the start
- **Reading at % change:** Absorbance reading at your chosen % change
- **Reading at peak:** Absorbance reading at peak
- **Reading peak-zero:** Absorbance reading at peak with baseline subtracted
- **Time to Peak from zero:** Time to maximum absorbance from the start
- **Time clotting to peak:** Time between the selected clotting value and maximum absorbance
- **Area under the curve:** Calculates the areas under the curves
- **Time at max rate increase:** First derivative, time at maximum positive change
- **Time at sign change:** First derivative, time where curve changes direction

The options that are calculated from the first derivative transformations are susceptible to noise and should be used with care. First derivate plots are not shown on this tab but are available from the second tab, *Curve*.

The plots above the Results table show graphically which selection is made.

Graphs, results table and setting table can be copied to the clipboard by highlighting and right-clicking using the mouse for pasting into an electronic notebook or OneNote or Excel, for example. If you download and run the ui.R and server.R files locally in RStudio, you can search for the line of code in the Results Table section of the server.R file that contains “clipboard” and remove the # from the beginning of the line. If this line of code is active, the contents of the table are automatically placed in the clipboard and can be pasted directly into another program for further analysis.

Select a parameter

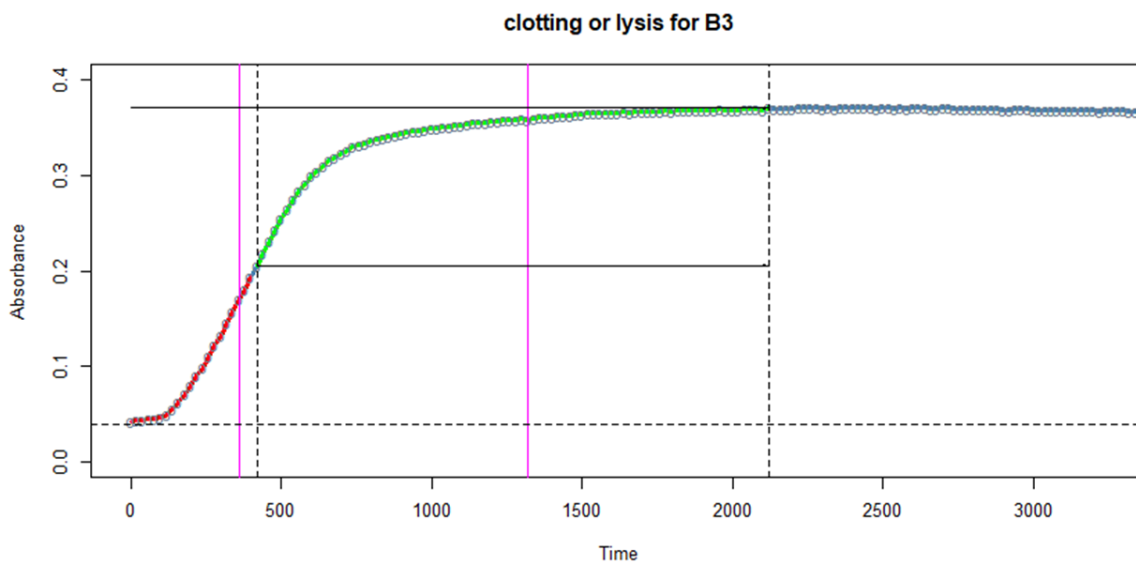
Column names
 chosen zero
 Time to % change
 Reading at % change
 Reading at peak
 Reading peak-zero
 Time to peak from zero
 Time clotting to peak
 Area under the curve
 Time at max rate increase
 Time at sign change

all.change.Time for 50 % of maximum

1	2	3	4	5	6	7	8
346.67	208.89	305.45	241.11	234.74	304.62	241.05	364.44
657.14	420.00	560.00	452.31	475.00	568.89	454.67	648.89
1135.00	780.00	940.00	837.50	810.00	996.67	808.89	1156.67
1800.00	1395.00	1650.00	1500.00	1500.00	1753.33	1525.71	2005.00

The *Curve* tab

The curve tab allows the user to focus on a single curve, which is selected from the drop-down box in the upper left corner. The plot includes lines corresponding to various analysis selections available. The radio buttons under **Selected Results** specify what is shown in the table below the graph - all results, or results from the first derivative plots of time at maximum increase or time at sign change. As mentioned above, these results are susceptible to noise and should be checked by careful visual inspection alongside the curves.



The *All Results* tab

Here there is a table of all the values from each well for each parameter available. It is possible to click through the data using boxes at the foot of the page and to show data from start to end or in reverse.

The *Raw Data* tab

On this tab the name of the data file loaded is shown and the time and absorbance data. If fitted curves have been generated, the new data will be displayed with additional time points.

The *Explore* tab

This tab provides a simple opportunity to explore your results graphically. The default plot is a heatmap, so you can see patterns and extreme values. If a scatter plot is selected you can select what should be plotted on the x and y axis to investigate relationships between various parameters. Points can be identified by hovering over them with the mouse.

The *Settings* tab

Here a table of settings is provided summarising the settings used in the analysis, which can be copied for future reference to aid reproducibility.

The *Help* tab

The *Help* tab summarises these help notes and provides citation details.

R code, data and help files are available on github

More information on Shiny apps and links may be found at Shiny-clots