

Data analysis in MyAssays with Cubic Spline

In this Technical Note, you can find information on how to use MyAssays and learn more about how to analyze data using the Cubic Spline

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Introduction

A curve fitting model is needed to determine the concentrations of samples after measurement with Mercodia's immunoassays. Among the different curve fitting models available, the Cubic Spline is a curve fit suitable for calculating concentrations from sigmoidal calibrators.

For this model, the inputs are the raw data signals from the reader, the already determined concentrations of calibrators and the chosen curve fitting model.

The algorithm uses the inputs to make systematic guesses and arrives at parameter values that best describe the calibrator points on the standard curve. This is done so that the distance to the points and the curve is as small as possible. The outputs are a standard curve and the calculated concentrations of samples in the assay. Figure 1 shows a schematic process of curve fitting.



Figure 1: Schematic process of curve fitting.

MyAssays

There are different programs where you can evaluate the results you obtained from your reader. Most of Mercodia's products are validated using either Magellan (Tecan) software or MARS (BMG Labtech). If you do not have access to these programs, you can make use of MyAssays which is a free-to-use online tool used by many organizations.

There are however a few things you should keep in mind when using MyAssays:

- Mercodia's products are not validated using MyAssays.
- Mercodia's products are not validated with blank reduction. Therefore, please <u>do not include</u>
 Calibrator 0 in the standard curve when using MyAssays. Specify that the negative control (calibrator 0) equals to zero for the blank in the plate layout. The reason is that blank is obligatory to add in the plate layout in MyAssays.

Different programs have different algorithms, which means that even if you use the same curve fitting model with the same data input, the output parameter values may differ from program to program.



Step-by-step guide on how to use MyAssays

- 1) Open an account at MyAssays.com
- 2) Search for "Cubic spline" on MyAssay "Search box" and that tool will open.
- 3) Add your measurement data, which is the raw data from your reader output. Remember to specify that the negative control (Calibrator O) equals to zero. Use the dot "." as the decimal separator.

Cubi	c Spli	ne		Q										
genera	l curve	fit Cu	bic Sp	line										
Quantitative analysis of samples using a Cubic Spline.														
All samples are first corrected by the mean of the blank group measurements. The standard data points are plotted (concentration vs. corrected measurement) and a Cubic Spline is applied to these points. The concentrations of the unknown samples are determined from the fit.														
For mea	surements	s outside	the ra	inge of th	ie standa	irds a line	ear extra	polation	is used.					
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4	Sam	ple ID)s											1
5	Run	Notes	5											1
Calculate														



4) Adjust the Microplate so that it corresponds to the microplate set up out of your experiment. Yellow for blank, red for standards, and green for samples.

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5) Fill in the standard concentrations (Calibrators). The concentrations and units should be stated on the standard vials

3	Standard C	Concentra	tions
	Review or edit the	e concentration	values here: 🔞
	Standard	Conc.	
	Standard1	0.197	Series
	Standard2	0.504	Series
	Standard3	1.54	E Danta
	Standard4	3.12	Paste
	Standard5	6.72	

6) Add Sample IDs if you have any.

Sample IDs

4

Comunito	10	
Sample	10	
Unknown1	Low	
Unknown?	High	Series

7) Add run notes if needed and press "Calculate".

5	Run Not	tes	
	Optionally p	rovide additional data to store alongside the results for this assay:	
	Run Name:	Test of low and high controls	0
	Notes:		•



8) Click on the results file.

1 Measurements	1
2 Microplate	1
3 Standard Concentrations	1
4 Sample IDs	1
5 Run Notes	
Optionally provide additional data to store alongside the results for th	is assay:
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• January 41,82 1.0 13585 0.03	
• Annexe 2 (3, 11) 57 3355 0.255	



9) Here you can evaluate your data. You can find the measurements of the standard curve and the concentrations of calibrators. The concentrations of controls and samples will be expressed in the same units as the calibrators (units used in point 5). You can also export the data to Excel.

