

Belysa: A Curve Fitting Software Webinar Q&A Answers

**Immunoassay Platforms Solutions Webinar Miniseries
Part 1, Webinar #1**

**Field Applications Scientist Team
Lawrence Rentoul
April 2020**

Brief Description

This document provides answers to a Question and Answer series from a webinar, **Belysa™: A Curve Fitting Software**, held on April 2, 2020.

Is this software GLP compliant?

No. At present, there is not a 21 CFR, Part 11 compliance module built in. This is possible in a later module, but not the RUO version.

Will we get a copy of this presentation?

We will make this available online at a future date. Once it is available, we will reach out to all attendees and registrants.

Can the Belysa™ software handle Bioplex Manager files?

Yes.

Can I copy and paste dilution information for each sample?

Yes, but it is better to just reassign the unknowns in the plate map and enter the dilution factor there. If a single well needs changed, it can be done in its free text field.

Can the Belysa™ software handle 384 well plates?

Yes.

How does Belysa™ handle ELISA data when data formats are different for every vendor?

The data is always very simple and a generic file input identifies the plate format (e.g., 96 well) and then aligns the O.D. reading with A1, A2, etc. You can then annotate the values in the Belysa™ plate map.



Can you flag low bead count as <35 in the MFI view?

In the raw data table, you can flip between count and MFI. Then, you can rule put the count-based results. It is not the same page, but right next door!

In general, flag rules can be added, removed, or edited via Tools->Settings->Auto-Flag

If my LLOQ/ULOQ values are defined differently from what the multiplex assay recommends, can I adjust these values in the software?

Those values are specific to the assay that you have run in your laboratory at that time. Anything from a vendor is only indicative of achievable LLOQ/ULOQ, e.g., the LLOQ requires <20% CV, +/- 20% recovery bias, and that the points above is also confirm to these criteria. This can be affected by your matrix being studied and the tightness of the replicates in the assay.

Can Belysa™ use the QCs to batch correct across multiple plates assayed over time?

No. The parallelism and relative potency functions allow you to confirm similarity of calibrator performance and the line equations are reported in an output file; however, there is no calculation function to “normalize” results.

What exactly is a Robust 4PL versus 4PL?

The curve equation is the same, but the numerical procedure to fit the 4 (or 5) parameters has similar robustness to potential outliers in the standard data.

Does this software work on both PCs and Macs?

PC – yes it is Windows compatible, including the latest Windows version (Windows 10). It is not Mac compatible using MacOS. We have had groups use a Windows emulator on Mac computers and it worked fine. In the future, we would like to be compatible in both operating systems.

Can I define control samples that are being re-run for longitudinal studies and use those to control assay performance?

You can set them up as their own defined group and label them as such, Then, check the reported results against other assays.



Do you think in the future the “results” units will become a dropdown menu? And when changing things like expected standard concentrations or dilution factors could there be an option to change it for all analytes at once?

You can change units in the software, but not as a dropdown menu at the moment; but yes, it can be arranged. Currently, there exists the ability to change standard concentrations all at once.

Is there a way to lower the LLOD? Because in our lab when we used Belysa™ (new users) and it seems that LLOD is drawn based on data point is high even if the standard curve looks good.

We are not aware of the LOD going that high. The LLOQ can go up the curve and that is driven by >20% CV and/or recovery bias greater/less than +/- 20%. If that is corrected, then the LLOQ will drop further down the curve.

Can I turn off the display of the LLOQ vertical line?

Not at this time.

Can Belysa™ compare 4PL or 5PL automatically and provide the best fit?

Yes, that is the purpose of the optimization function. You do have the option to select a preferred fit.

It seems like Belysa™ favors the Robust 4PL or 5PL, why?

We have seen mostly 5PL, but that is due to more instances of viewing MILLIPLEX® assay data.

A single dongle allows only one user to work on Belysa™. Can you provide two dongles for high flow laboratories, or is this an additional cost?

Currently, this requires the purchase of two copies of Belysa™.

Can Belysa™ handle MSD and Simoa data output?

Not currently, but we are exploring that as an option. Our goal is to make Belysa™ platform agnostic.



Can standards, generated curves, and QCs be transferred across plates for batch runs?

Yes, you have to create a new experiment in Belysa and import the external curve. Then, it can be compared with all the batches run without Standards or QCs.

Does Belysa™ automatically import dilution factors?

Yes, if the dilutions were entered in the xPONENT, they will automatically be “captured” in Belysa.

Can I see all results in the 96 well plate form?

Yes, results can be exported in 96 well format.

Can Belysa™ manage data normalization? Normally when we run qPCR based assays, we have to normalized the target data to the internal control so there are two curve fitting steps (target and control), followed by data normalization, then curve fitting.

No. Belysa™ confirms method consistency rather than data normalization.

Are you planning to include “virtual” dongles at some point? In my experience, dongles get inevitably lost.

Not for this version, but we are aware of the limitations and it was a matter of discussion internally. This was the easiest way of keeping the product flexible. There are many barriers with all security option., i.e., physical, virtual, cloud, etc. We plan to explore virtual dongles in the future.

Is data hygiene the same as data integrity? Does Belysa™ have an audit trail feature?

Data integrity sounds like the same thing! At the moment, Belysa™ records the changes that you make and puts them in the output files. However, this is not secure. Calling it an audit trail would not be correct.



Does parallelism work with optimized curves?

Yes.

Can you explain what PL is?

First, there is a number before that (4 or 5, typically). Then, it stands for Five Parameter Logistical curve fit.

As one of the key developers, what feature of Belysa™ are you most proud of?

The curve comparisons. It is a very powerful tool. Getting confirmation that your method is consistent and being able to prove it is extremely useful.

Is it possible to purchase additional user dongles?

Yes, but one must purchase the entire Belysa package.

How is LOD calculated by Belysa™? 2D/3D or 20%?

$LOD = \frac{3 \times \sigma(\text{blank sample})}{\text{Slope}_{\text{I}}}$, where Slope_I is the slope of the least squares regression line that best fits the first three standard groups with increasing concentrations starting from the blanks.

Can you organize the data if I just want to see the flagged data?

You can't organize your file to see just flagged data, however, there is "Excluded Wells tab" that displays all the wells and analytes that have been excluded from results calculation for any reason.

Can you export the data so that the individual analyte results are in order of your unknowns?

You can export the data into Excel and sort by your unknowns. You can also assign unknown samples into different unknown groups, so that exported data will be sorted by sample group

Can you assign dilution factor on different data groups on the same assay?

Yes, a dilution factor may be entered as you mark different groups of unknowns or with a free text field for that specific well.



Can you post the link for future patches.

Yes. Follow the link below to register, then you will be notified. It will actually be a complete software. You will uninstall the old and replace it with the new. Your data remains unaffected and software upgrades will not affect operation of the the USB dongle.

<https://www.sigmaaldrich.com/life-science/immunoassay-platform-solutions/curve-fitting-software-download/register.html>

How do you turn on the LLOQ line?

It is always present.

Is there a trial you can offer?

No, unfortunately we do not have a website set up to monitor that and we do not want to offer a stripped-down version. Our Field Applications Scientist (FAS) Team can work with you to analyze data during a demo period. These have been successful with a variety of customers. Please reach out to any contact at MilliporeSigma and they can connect you with your FAS.

How do you see Belysa™ developing over the next year or two?

We will increase the flexibility, e.g., change %CV or recovery informing LLOQ and even make it biomarker specific. There will also be further confirmatory tools and the start of data analysis (statistical) capabilities.

Are you able to removed standard points manually, depending on the analyte of interest?

Yes.

What generation is the current Belysa™ software?

We are at V1.0. We expect to release V1.1 in the coming weeks. All software updates are free!

You mentioned about the calibration kit when we want to compare different batches of the kit. Is it now market available?



We produce an excess of calibration material (the protein in the standard curve). Should you require extra, your Immunoassay Platform Specialist or FAS can help you. We do not sell it over the website; rather, it is done through our field-based team.

Can QCs be included for mathematical comparison between runs in addition to standard curves?

We can look into that. I also think this would be valuable for researchers using a plate-to-plate sample strategy. Consider it on the list for potential updates.

If I optimize the standard curve in the single analyte view, does it optimize the standard curve of all other analytes?

It depends on the call you make. You can exclude a full well, but also individual analytes of that well.

The "Optimize" function will optimize standard curve fit for the current analyte, and you can additionally choose the "Copy Fit to All Analytes" function to then apply that same fit algorithm for all analytes subsequently

