CombiStats Online – Training

Q&A session

17-21 November 2025

Module 1: Introduction to the online application

Question Asked	Answer Given
Is it possible to share files with people from another organisation who also have a CombiStats licence?	Files on a given workspace cannot be accessed from a different workspace. If you like to share files with another organisation, you could export them and then send them manually (e-mail or shared drive).
Does automatic deletion apply to all saved files or just inactive ones?	Automatic deletion 3 years after the date of creation applies to all files (active or not). Templates are not deleted, but these files do not contain experimental data, just data layouts and analysis options. Note that users are informed before files are sent to the trash, so they can export them if they want to archive them.
Can I have more than one table of blank results so that the means and SD can be calculated independently?	No, there is only one table of blank results.
Could you explain again how to obtain the extra columns with mean, SD and RSD?	In the menu, click on *Raw data* and select *Show statistics*.
Is it mandatory that each user has their own "seat" (in the same workspace)? I am the head of the laboratory and I do not perform any analyses but I have to review the results. If there are 2 people performing the analyses in my lab, do we have to buy 3 seats?	Yes, you also need a seat if want to access the working file. If you review the results based on the PDF report, then you do not need a seat because PDF reports are always exported locally.
Is there any trace or record in the software that would be available during an internal or external inspection? Are analysis results automatically saved in the system, and how are they archived?	Analysis results are indeed saved in the system and it is possible to restore a previous version (check the *Revisions* panel on the right hand side of the application). Note that these versions are available for 3 years from the date of creation of the first version.
Can you show where we can access the "Group" option? It is a very useful feature for me and my colleague.	This was shown during the presentation and is explained on page 13 of the User guide.

I want to check or confirm that the application works and calculated everything correctly at my end. Can I do this only by running an example template that you have provided?	That is right. If you would like to do some checks at your end, you can run some examples in the CombiStats interface and compare the outputs with the printed results.
Could you please explain the conversion from Excel to .epax again?	This was explained during the webinar.

Module 2: Assays based on quantal response

Could you please explain what the *Preparations* row in the ANOVA table represents?	In the ANOVA table, for a PLA for example, *Preparations* represents differences between the regression intercepts. It is not necessarily an issue if there is a significant difference. Indeed, if the potency of the test preparation is expected to be much lower or higher that the potency of the standard preparation, a significant difference will be expected in the *Preparations* row of the ANOVA table. For more information, please see section 3.3 of the following document: https://combistats.edqm.eu/faq/link/97/
Is there a way of combining assays before publishing?	No, files should be published first. Publishing means saving the results of a file (considering that they are the final results that you want to combine).
Can the estimated potency of the sample be *calculated* within the software? I am using a 6x6 Latin square.	Could you please send us an example to EDQM Stat (Stat@edqm.eu)?
Will there be a session that uses Latin square examples?	No, the training sessions do not contain any examples using the Latin square design. However, information about assay designs can be found in section 1 of https://combistats.edqm.eu/faq/link/97/ and section 4 of https://combistats.edqm.eu/faq/link/79/.
When replicates are very close, sometimes non-linearity is significant whereas the results appear satisfactory (using qPCR for example). Is it possible to correct this effect?	Yes, please refer to the decision tree suggested on page 38 of https://combistats.edqm.eu/faq/link/97/.
How can I calculate EDs other than ED50 (e.g. ED80) with CombiStats?	You can change the percentage in *Advanced options* > *Effective dose* from 50 (default) to 80. If you would like to get an ED for various percentages, you can also use *Advanced options* > *Y values* (for example, enter 0.2, 0.5 and 0.8 to calculate ED20, ED50 and ED80).
Can we use the effective dose estimate to determine whether test sample potency is consistent? Batch to batch has similar potency, it is usually used to assess pyrogens for the monocyte-activation test.	CombiStats will provide the estimated potency for the test samples compared to the standard.

Can there be only a star on the non-linearity or non-parallelism table?	According to Ph. Eur. general chapter *5.3*, the non-parallelism and non-linearity effects should be non-significant (no stars). You may have additional specifications in your SOPs on how to proceed if one star is present in the ANOVA table. The interpretation of the stars is as follows: - $p > 0.05$: no significant effect; - $p \leq 0.05$: significant effect (*); - $p \leq 0.01$: highly significant effect (**); - $p \leq 0.001$: very highly significant effect (***).

Module 3: Assays based on quantitative response

Question Asked	Answer Given
When running several test preparations vs a standard in the same experiment, is it better to analyse each test preparation vs the standard separately or analyse them all together? I find the 2 options give slightly different results in CombiStats (e.g. for potency).	That is right. Some elements of discussion are available on page 26 of the following document: https://combistats.edqm.eu/faq/link/97/
If the slope ratio of a sample with a standard in PLA analysis is 1.0, does it mean the curves are parallel and a ratio of 1.0 can replace the acceptance criteria for non-parallelism?	If the ratio = 1, the slopes are perfectly equal! This is usually unlikely, so it would be better to set acceptance criteria to values lower (- theta) and higher (+theta) than 1.
What is the difference between non-linearity and non-linearity table 1 and table 2. Which should be taken into account for assay validity? All 3?	The non-linearity contrast is tested taking into account all data or only the data from table 1 and table 2. If the non-linearity criteria is met for all three, the assay is valid. Non-linearity for only one of the preparations may indicate an issue. How to deal with this would usually be specified in your SOPs.
In equivalence testing, should the *p*-value of non-parallelism still be taken into account for assay validity?	You can decide if the parallelism evaluation should be based on the *p*-value or on the equivalence testing. The approach is usually fixed before performing the assay.
Should the log-transformation be done, decided on during assay validation, described in the SOPs and then done for all assays, or can I decide when I see the data of an individual assay if a log-transformation is needed or not?	It should be decided during the assay validation phase and specified in the SOPs if a transformation should be applied. It could be described as well whether another transformation can be used.

How can it be that the non-linearity *p*-value is valid (0.09), but the non-linearity of table 1 is not valid (standard) (0.03) and the non-linearity of table 2 (sample) is valid (0.67)? Should the assay be accepted for linearity?	The overall *p*-value (0.09) is not much higher than 0.05, so it is not a big surprise to see that it is lower than 0.05 for one preparation and above 0.05 for the other. You could detail the decision rule in your SOPs. For example: if the overall *p*-value > 0.05 and individual *p*-values > 0.01, then the test is valid; investigate individual *p*-values ≤ 0.01; allow (or not) the exclusion of data points; perform robust analysis instead. Note that Finney (1971) says that such analyses are instead robust to moderate deviations from linearity. It may be worth trying to relax the significance threshold a little bit.
Can I simply try different transformations as I want to? How does it depend on the kind of analysis (PLA or 4-PL)? Are there limitations? Since it could also be a clue for something that went wrong during the assay, would I mask them by choosing another transformation?	In the method/assay development phase, a large signal-dose range is analysed, and the dose-response is characterised. In this phase, the dose range and analysis options, including transformation, can be fixed for the routine testing. You are right, the transformation should not mask any issues with the assay performance.
The sigmoidal curve shown in the presentation for the 4-PL analysis appears not to have classic upper and lower asymptotes. Are asymptotes like that sufficiently described for an assay or are the values for the asymptotes excluded/not shown in the example? It does not seem to have 1-2 points in the asymptotes as mentioned in the presentation.	It is true that one additional data point in the upper plateau could be expected. We will review the example, thank you.
I always had in mind to use the logarithmic transformation for an *in vitro* bioassay, but without really knowing why. As I understood from the presentation, it is better not to perform any transformations for the analysis for *in vitro* assays and to only do so in case of difficulties with the residual plot as described in the presentation. Is this right?	From the assay development, you should be able to say if a data transformation will be needed in routine testing. Then, you will use it consistently in routine assays. It could be that no transformation will be needed, in which case you will have to state in your SOPs if a data transformation is allowed in the case of an invalid assay.
If I have a zero-dose, do I have to select the slope ratio model?	Yes, the zero-dose cannot be part of the PLA, because the x-axis = log(doses) and log(0) = Error. Make sure that the other doses in your assay are arithmetic, e.g. 0, 10, 20, 30, etc. (dilution step: +10).

If we close a value on standard table, do we have to close on the sample table as well?	By “close”, do you mean delete? If so, I would say not necessarily. Assuming the data are independent (as said in slide 9 of the presentation), an atypical value observed for the standard should not have any influence on the other values.

Module 4: Single-dose assays, combination of assay results

Question Asked	Answer Given
Who can I contact for assistance when using CombiStats?	Send us your questions via the EDQM HelpDesk https://go.edqm.eu/hd.
Can the pooled estimate be expressed as a percentage?	The combined mean can be expressed as the % of an assigned/assumed value, provided that the assigned/assumed value is the same in each of the files to be combined.
Do you have any practice examples?	A series of examples is available at https://combistats.edqm.eu/faq/link/96/.
The example currently being shown is erythromycin. In our lab, we make a calibration curve for the standard, but I see in your example that this is not necessary and just a regression line would suffice. Where I can see that we perform the assay without a calibration curve??	I would rely on what is indicated in the monograph. I do not know it by heart and would need to check.
What I mean is: is it written in any chapter in the pharmacopoeia that the potency assay of erythromycin can be performed with just a regression line?	I do not have the answer right now. I would suggest that you send a question via the HelpDesk.
We performed the assay using a calibration curve, but when we do the analysis this way in CombiStats, we do not get the same results as we do when we use Excel for the statistical analysis.	Could you send us an example to Stat@edqm.eu so we could try to see why you get different results with CombiStats and Excel?