CombiStats Online – Training

Q&A session

10-14 March 2025

Module 1: Introduction to the online application

Question Asked	Answer Given
Can only LMs make a file for read-only mode? Or all users?	Anybody can make a file read-only.
How can I add a table for blanks?	In the Wizard options, select *Show blank results = Yes* and then indicate the size of the table of blank results.
Is it possible to increase the number of seats we have between yearly renewals?	Yes, via the EDQM store > CombiStats seat extension.
Is there a reason why you changed to R^2 instead of providing |r|?	This was a request from users. R^2 appears to be more commonly reported than r (which will not be shown in next release). However, the square root of R^2 gives the r value that was provided by CS-Desktop.
Is it possible to enter potencies (e.g. IU/mL) for the doses of assumed potency?	Yes, as in the previous version. These assumed potencies should be entered in the Preparations table.
Is it possible to exclude individual graphs from the report?	In the next version, only the graphs displayed on the interface will be shown in the PDF report.
The report is too long now; will there be a one-page summary in the next software version as was previously the case? When will this updated version be released, roughly?	The next version will be released at the end of April 2025. On average, the report will have 3-4 pages.
Will there be a certificate of attendance at the end of the webinar?	We can issue a certificate upon request: please send an e-mail to events@edqm.eu.
Is it possible to provide the full demo Excel workbook with macros for automated data entry from Excel for review and learning?	Yes, it will be posted on the web page for this training session.
Are templates also deleted after 3 years?	No, the templates will not be deleted automatically.

Is it possible to show the individual curves of the samples like in the desktop version?	Not directly: you would need to manually exclude results of the other data tables to achieve this.
How will users be informed about updates and how long in advance will the new update be announced?	A message will appear about 15 days before the next version in a yellow banner on top of the web page. The list of changes will be listed in a separate note for guidance (Help page). On the day the new version is released, the list of changes will be listed in the release notes.
Do we have to validated CombiStats after every update?	The EDQM validates each new release. However, you are not prevented from performing checks specific to the kind of analysis you routinely perform.
Has it been mentioned if the number of pages of the report will be reduced in the next version? There to many page breaks.	Page breaks will be removed.
Have you performed any qualification tests for this new online app?	Yes, the EDQM validates each new release.
When we add concentrations for doses for assumed samples, we get the message "doses cannot be reported in IU because potency is assumed". Is this right?	That is right. As the potency is only assumed (not known), doses cannot be entered in IU.
Can an export be done with only the statistics and without the graphs or audit trail?	This will be possible in the next version.
The speed of the online version is very slow. Will it improve in the future?	Not all users are experiencing this issue. We will try to identify the possible cause.
Some data are now combined: for example, LLC, LLU *27.4332 (26.0496, 28.8168)* all in the same cell. Is this a problem with our template?	No, it is true that in CS-Online, the point estimate (e.g. potency estimate) and associated confidence limits are all in a single cell.
When integrating multiple results from repeated tests, non-linearity is expressed on a per-sample basis. However, why are the coefficient of determination, regression, and non-parallelism expressed as a single value?	Those statistics are based on the global model performed on the results of the samples. With regard to non-linearity, a global contrast is also calculated in the ANOVA and then split by sample.

Is there a demo version of the software that we can try ourselves?	There is no demo for CombiStats, but we recommend that you read the notes for guidance (accessible even if you do not have a licence) as they contain many screenshots and explanations about CS-Online.
Once an assay has been defined, can it be saved as a template?	Yes.
Are the copy and paste options available?	Not for all tables. They are available for the raw data tables and the design/observation tables, for example.
Can you rename 'Rep1', 'Rep2', etc..?	No, this is not possible.
Where do you indicate the dilution step?	Predilutions can be indicated in the Preparations table (table above the data tables).
Why cannot we rename doses if we set it to automatic? This option is only available in the manual setting.	To save time, you can generate doses automatically, then revert to *Dose entry = manual* and change the required doses.
Are the parameters set in the Wizard available when printing the report?	Yes, because these are the analysis options, and it is important to know them in order to understand the statistical results.
When importing data from the desktop version, the potency estimation information does not match the current data.	Please send us this example (via the HelpDesk: https://go.edqm.eu/hd).
Even though you cannot provide a certificate or qualification report, is it possible to obtain a statement from the EDQM that the desktop version has been changed and all the software has been moved to this online version with the corresponding validation process?	Does the statement available on the last page of the release notes answer your question? https://combistats.edqm.eu/faq/link/87/
Can we export results to our desktop and open them later?	Yes, you can export a file (e.g. .epax) locally and open/reimport it later.
Can we open our desktop version data using the online version?	Yes. You can import local files, including files from previous versions of CS-Desktop (v 5.0-7.0).
Can we get a recording of this training session for future use or for those who missed this session?	Recordings will not be shared for this session, but the slides will be made available.
Can you offer any qualification protocol of the new online system? Have you performed any qualification tests for this new online app?	No, sorry, the EDQM does not provide qualification protocols/reports.

The system for automating the data transfer that you showed is based on macros and exported files that can be modified in the process. This is not compliant with data integrity and would be tricky to validate. Are you working on a more robust connection (not Excel based) with other systems (inhibition hallus measurement systems, for example) for future versions of the software?	The purpose of this Excel-based demo was only to show that automation is technically possible. Of course, you should adapt it to your own systems and constraints.
Is it one licence per user or can multiple users share the same licence (but not log in at the same time)?	One licence key corresponds to one workspace accessible to several users.
What distinguishes individual users from group users?	Groups make the management of folders easier. By sharing a folder with a group, you share it with all the group users in one action. Otherwise, you would have to share it with each individual user.
If a licence consists of 7 accounts, can the group only consist of 6 people?	When assigning users to CombiStats, one seat is already taken by the licence manager. This means you can only assign 6 additional users to the remaining 6 seats.
For example, a licence with 3 access users means that only 3 people can work with this licence. Is this correct?	Yes. A licence manager can remove one access from one person and assign it to someone else, if needed.
Can you adjust the time indicated on the first page of the CombiStats data file?	Not yet, but it will be possible in the next release.
It was confirmed that the CombiStats programme desktop version will end its service on 2025.03.31. Does this mean I can no longer use the desktop version?	CS-Desktop can be used until end of this month.
When multiple samples are calculated at the same time, only one non-parallel and regression data value is calculated, whereas non-linearity is calculated by the number of samples. Is there a reason for this?	A common slope model is indeed calculated, according to Ph. Eur. general chapter 5.3. A prerequisite is parallelism of all individual slopes (1 test). However, individual slopes are available in the table just below the ANOVA table and compared against the slope of the standard preparation (differences and ratios vs. slope of the standard are reported).
Can we add IU/mL units to doses?	Doses can be entered as dilutions (e.g. 1/1, 1/2, 1/4, etc.) or as concentration contents (e.g. 100 IU, 50 IU, 25 IU, etc.).

Module 3: Assays based on quantitative response

Question Asked	Answer Given
When we have several samples on the same plate: is it better to perform one CombiStats calculation sheet with all the samples or independent calculation sheets for each sample?	This is an important question to consider because the potency estimates and 95% confidence limits calculated for the test preparations will differ depending on the type of analysis performed. The global analysis would be more appropriate from a statistical viewpoint, as it reflects the experimental design (all preparations tested in the same run) with some tangible benefits: i) control of false discovery (type I error) rate; ii) robust estimates of slopes and intercepts; iii) robust estimate of assay repeatability. Despite the elements above in favour of the global analysis, one may decide to perform separate analyses (one analysis per test preparation), depending on other considerations, including: i) there may be a formal request from a regulatory body to perform separate analyses; ii) separate analyses can result in a more flexible quality control process (e.g. invalid results of one preparation will not affect the processing of other preparations); iii) the analyst may be testing unknown products with no guarantee of similarity between them. Using separate analyses will make it possible to evaluate each individual preparation.
Shouldn't the y transformation be predefined as part of the SOP?	Yes, this is recommended. During the development of the method, a transformation is chosen to best describe the dose-response relationship.
Regarding parallel line analysis, we often encounter problems with *p*-values < 0.05. How it this value calculated and affected?	The better the repeatability (variance between replicates), the lower the *p*-value. Try to see whether the low *p*-values are due to very good repeatability, in which case an alternative approach for assessing linearity and/or parallelism may be envisaged.

What is the difference between model plot and average plot?	Model plot is the regression line where the slope and intercept are calculated on the linear model. The average plot is just the mean value per dose.
What is the difference between the linearity test and the R^2?	The linearity test is a lack-of-fit test while R^2 is the proportion of the variability in y-values explained by the selected model (even if this model is not necessarily the model that best fits the data).
What is the impact if the doses for log-transformation are not equidistant? For instance, if my doses are 1/40, 1/60, 1/80 instead of 1/40, 1/80, 1/160?	The dose range of your observations is not the same. The regression analysis is based on the lower dose range in the first case. Even if the doses are not equidistant, CombiStats performs the calculations. However, equidistant doses over an appropriate range would better represent the dose-response relationship.
Does it make a difference if I put "? IU/ml" or for example "100IU/ml" for the assumed potency?	No. If you enter a known potency for the assumed potency, the relative to assumed potency will also be calculated and displayed.
What is the difference between R^2 and r from a statistical point of view?	The correlation coefficient is the square root of R^2: $r=\sqrt{R^2}$.
If I try to insert large reportable values (like 6x10E9), CombiStats will not accept it unless I write all the numbers. This was not the case in CombiStats Desktop - should I import the data in a special way?	If all the results are that high, I would recommend removing some zeros by dividing all the results of all the preparations by a constant (e.g. 1 000 000). It will not affect the calculated results, but the iterative process (to convergence) will benefit from this.
How should one choose a proper transformation for the data?	You can observe the dose-response relationship and distribution of responses at each dose over the dose range based on an average plot. For example, if the plot is curved as an exponential function with increasing variability over the doses, a log-transformation would make the dose-response curve linear.

What are the different weighting options and which one should I choose ?	Data analysis using the slope ratio and parallel lines models is based on the principles of simple linear regression. In particular, it is assumed that the variance between replicates is stable between doses (unweighted regression). The robust regression (Huber's weights) option may be useful for atypical results where the influence of the regression parameters on the calculated values is reduced. For 3-parameter models (exponential curves) and 4- or 5-parameter models (sigmoid curves) applied to quantitative data, it is also assumed that the variance between replicates is stable between doses (unweighted regression). However, if this increases with the signal, a weighted regression ($1/m^2$) may be considered. The Poisson regression (1/m) can be used for count data (e.g. CFU/PFU). Finally, the *Weighting > User-defined (w=…)* option can be used to apply a custom weighting to the data.
Can we use the "user-defined (s2) variance" (based on historical data) to obtain a *p* value higher than 5%?	The variance based on historical data can be used instead of the variance of the current assay, when the latter is unexpectedly low. Usually, assay variances are monitored using a control chart and lower variances are those below the lower control limit.
For 4PL models, what other tests can be performed if the assay fails in linearity or parallelism?	An approach proposed by Bliss may be useful in case of non-linearity. For parallelism, an equivalence testing approach may be envisaged.
Is it possible for the model to be linear when one of the samples is not? If so, how can this issue be addressed?	The overall non-linearity contrast in the ANOVA table is further split by sample preparations. When the overall contrast *p*-value is close to 5%, one or more sample contrasts may be lower than 5%. The SOP method would tell you what decision should be taken about the assay in such a case.
Is it possible to perform other tests, such as the Bliss test, if there is a lack of linearity?	I think so. You can describe the conditions when this test is used in your SOP.

What should we do if we receive the warning during the calculation: no convergence reached after 50 000 iterations?	Convergence should be reached in a limited number of iterations. Usually, there are 2 root causes : i) atypical data entered; ii) improper model selection. You could send us an anonymised example via the HelpDesk and we could try to see what is going wrong.
If we have an outlier in our data, do we have to avoid this outlier? Can we consider the results valid with this outlier or not?	Not all outliers have the same effect on the calculated results. That may depend on their distance to the regression line, on their location (mid-dose, tails), etc. I believe that they may be kept in some situations, but a decision tree should be available in the method SOP to define the conditions for inclusion/exclusion.
If there is a star on the line of parallelism or linearity, is the assay acceptable?	The answer should be indicated in your SOP together with possible actions (to improve linearity and/or parallelism).

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

Question Asked	Answer Given
If I have multiple estimates, can I also calculate a simple average (arithmetic or geometric average) value? What is the difference between combination (weighted or unweighted) and average calculations?	You could, but if the number of assays to combine is small, you would end up with quite large confidence limits for the overall mean. Ph. Eur. general chapter 5.3 states that "The number n' of estimates of M is usually small, and hence the value of t is quite large." So if your assays are independent, it would be better to use one of the other 2 calculation approaches to get narrower confidence limits around the overall mean potency value.
For the potency value of the samples in some of the tables you illustrate, you add a value but it is the one I want to calculate. So why do you add the value 2.5 IU/dose?	For the multiple-dose assays, you may indicate a potency value (that you assume to be) for the test preparations. The potency of the test preparation is calculated against the standard, but you will also get the information on the estimated potency compared to assumed potency you indicated. For single-dose assays, the assumed potency is used to calculated the limit value.
Is a *p*-value less than 0.1 a guidance value?	For the evaluation of homogeneity of potency results, yes.
What is the statistic C?	A calculated value which is used in the calculation to the 95% confidence limits of the potency estimate. According to Ph .Eur. general chapter 5.3, this C-value should be close to 1. This is why we will report C-values in the next release of the application, so users will have the opportunity to check this requirement.
Do you first have to run a normality test and then choose between the t or Mann-Whittney test?	You do not need to run a normality test. CombiStats performs the Wilcoxon-Mann-Whitney test by default for single-dose assays.
Is it possible that the decision of the weighted, semi-weighted and unweighted calculations could be pointed out by CombiStats?	No, because we do not know if the assays to be combined are independent or not. If they are not independent, the 3rd calculation method (general chapter 5.3, section 6.3. Unweighted combination of assay results) should be used.