Question: Which notation for the doses should I use, and how does CombiStats interpret them to perform the calculations?

Answer: This is perhaps the most difficult question about CombiStats to answer. Doses can appear in 5 different notations: Symbolic, Concentrations, Volumes and Dilutions (as ratios or logarithmic). For a better understanding of the internal representation it is helpful to introduce the concept of the Basic Dose (BD), often called the stock-solution depending on the way it is used. The BD is the dose as calculated on the basis of the information in the top section of the sample tables. The individual doses are calculated on the basis of this BD and the type of notation used for the final dilution series.

Let us consider the following example:

| Standard | |
|---|---|
| Preparation | Ph. Eur. BRP Batch 1 |
| Ass. pot. | 4.3 log10 IU/vial |
| Reconstitution vol. | 1 vial / 500 µl |
| Inoculation vol. | 100 µl / well |
| Doses | (1) |
| -1.6 log10 | 8/8 |
| -2.2 log10 | 8/8 |
| -2.8 log10 | 8/8 |
| -3.4 log10 | 6/8 |
| -4.0 log10 | 4/8 |
| -4.6 log10 | 1/8 |
| -5.2 log10 | 0/8 |

The assigned (or assumed) potency (AP) is calculated as $10^{4.3}$ IU/vial = 19952.6231496888 IU/vial. Note that CombiStats always calculates in double precision (i.e. carrying 15 significant digits). Also note that potencies are internally represented on a non-logarithmic scale, even if logarithms are used in the notation. The BD is now calculated on the basis of this AP and the subsequent 'dilution' steps: 19952.6231496888 × 1/500 × 100/1=3990.52462993776 IU/well.

Note that the term 'dilution step' does not necessarily have to represent a real dilution; It is also used for reconstitution steps, unit transformations, and volume administrations. Also note that the basic dose does not necessarily have to represent a truly administered dose but that it could also represent a concentration (on the condition that equal volumes are administered to each experimental unit).

The individual doses of the dilution series are calculated on the basis of the BD and the information given for the individual doses. The table on next page gives a variety of examples. Note that the final doses are always calculated in content units (in this case IU) which is the unit that appears immediately before the slash of the assigned potency.

The doses for the test samples are calculated in the same way. A special case occurs when a question mark is specified for a test sample. In that case, the BD of the test sample is taken to be equal to the BD of the standard and an implicitly assumed potency of that test sample is back-calculated. If no standard is included, the BD is set to 1.

Note that symbolic notation in the above table applies to models with logarithmic doses only (e.g. parallel lines or probit anaysis). In the case of slope ratio assays symbolic notation is interpreted differently.

If the assumed potencies were exactly correct, the expected relative potency RP would be exactly 1. The estimated potency (EP) is finally calculated as EP = RP × AP. The BD does no longer play a role in this final step. Note that the RP in a good assay is as close as possible to 1.

Summary of abbreviations: AP=Assumed or Assigned Potency. BD=Basic dose. RP=Relative potency. EP=Estimated potency.

| This notation… | …is interpreted as | Comments |
|---|---|---|
| -1.6 log10 | BD × $10^{-1.6}$ = 100.237446725454 IU | Logarithmic notation to the base 10 |
| -4 log | BD × $10^{-4}$ = 0.399052462993776 IU | Logarithmic notation. No base is specified, so it is assumed to be to the base 10 |
| -4 log2 | BD × $2^{-4}$ = 249.407789371110 IU | Logarithmic notation to the base 2 |
| 1/1 | BD × 1/1 = 3990.52462993776 IU | Ratio notation. Undiluted with respect to the basic dose |
| 1/10 | BD × 1/10 = 399.052462993776 IU | Ratio notation. Diluted by a factor 10 with respect to the stock solution |
| 2.5/0.5 | BD × 2.5 / 0.5 = 19952.6231496888 IU | Ratio notation. 'Concentrated' by a factor 5 with respect to the stock solution. |
| 3 IU | 3 IU | Concentration. Taken 'as is'. CombiStats now assumes that you diluted the sample in such a way that you obtained exactly 3 IU. Note that the basic dose is completely ignored in this case. The RP is directly applied to the AP. (In this example 3IU is equivalent with 1/1330.17487664592). |
| 2 well | BD × 2 = 7981.04925987552 IU | Volume units. In this case it does not seem to make sense, but it could be useful if different volumes are applied to the experimental units in the case the BD represents a concentration. |
| S1 | BD × $step^{1\text{-}row}$ = BD × 20 = 3990.52462993776 IU (if it occurs on the first row and the dilution steps are specified as decreasing with a factor 2) | Symbolic notation. Note that the first row is considered to be the basic dose (undiluted with respect to the stock solution) |
| S1 | BD × $step^{row\text{-}rows}$ = BD × 20 = 3990.52462993776 IU (if it occurs on the last row and the dilution steps are specified as increasing with a factor 2) | Symbolic notation. Note that the last row is considered to be the basic dose and that all other doses are considered to be weaker. |
| S1 | BD × $step^{1\text{-}row}$ = BD × $1.6^{-2}$ = 1558.79868356944 IU (if it occurs on the 3rd row and the dilution steps are specified as decreasing with a factor 0.625) | Symbolic notation. Note that a dilution factor of 0.625 is considered to be the same as a factor of 1.6 irrespective of whether doses are increasing or decreasing. |
| High | BD × $step^{row\text{-}rows}$ = BD × $1.6^{3\text{-}7}$ = 608.905735769311 IU (if it occurs on the 3rd of 7 rows and the dilution steps are specified as increasing with a factor 0.625) | Symbolic notation. Note that the 3rd row is considered to be weaker than the last row (the basic dose). |
| hello | BD × $step^{1\text{-}row}$ = BD × 20 = 3990.52462993776 IU (if it occurs on the first row and the dilution steps are specified as decreasing with a factor 2) | Symbolic notation. Note that you could use any string of characters for symbolic notation. The string has no specific meaning to CombiStats. Only the row on which it appears is important. |
| | BD × $step^{1\text{-}row}$ = BD × 20 = 3990.52462993776 IU (if it occurs on the first row and the dilution steps are specified as decreasing with a factor 2) | Symbolic notation. An empty string is also considered to be symbolic notation. |
| 0.5 ml | ERROR | This cannot be interpreted because 'ml' is not a valid unit in this example. It will lead to an error. The only valid units in this example are 'IU' (the part before the slash of the assigned potency) and 'well' (the part after the slash of the last pre-dilution step) |