PRECISION OF MICROBIAL ENUMERATION METHODS :

the teachings of CECALAIT's ringtests

In ringtests on microbial enumeration, the acceptability limits for precision cannot be derived from standardized values for repeatability and reproductibility, as most of the international standards for the enumeration of micro-organisms in food have not included such values until now. The determination of precision data began a few years ago in the course of a project, financed by the European Commission and for the time being, is finished only for *Bacillus cereus* and *Listeria monocytogenes*. In any other case, the acceptability limits are derived from values given in the literature or from desirable quality purposes. However, analysing a lot of results from several ringtests allows to determine the precision data of the methods. Then, it is possible to calculate the limits of their accuracy. These new calculated values can then be compared with the former limits and adjusting these ones may be considered.

Thus, Mrs ROLLIER used the results of several ringstests on microbial enumeration to determine precision data and to calculate acceptability limits. The methods she studied are the enumeration of microorganisms at 30°C (total aerobic mesophil flora), of coliforms and of coagulase + staphylococci. They all use **colony count** techniques.

In La Lettre de CECALAIT, table 1 reminds you **the present acceptability limits** used in CECALAIT microbiological ringtests. The definition of the parameters is given right under table 1.

Parameter đ is the mean of the differences between the true value and the results for each laboratory and for each sample. In the reports sent by CECALAIT after each ringstest, you can see that it allows to define two accuracy limits, symmetrical in relation to the zero difference. (see figures 3 and 4 in page 4 of La Lettre de CECALAIT).

As told before, these acceptability limits were derived from the literature or from desirable quality purposes in a given technical context. For instance, for the enumeration of the total flora, we used the same limits as the ones used for milk payment purposes. They correspond to the high quality grade required in interprofessionnal laboratories.

Observation of the results of ringtests

CECALAIT has been organising quaterly ringtests since 1992. They were first for the total flora and for the coliforms in milk. Since then, they also concern cheese and the enumeration of other microorganisms, including coagulase+ staphylococci and *Escherichia coli*.

So, more than ten ringtests could be used to estimate the standard deviations of repeatability and of reproducibility, respectively Sr and S_R, then to calculate the limit for đ.

The calculations follow the steps below.

Slab is calculated from the following standardized equation (see for example ISO 5725) :

$S_R^2 = S_{lab}^2 + Sr^2$

Slab is the between laboratory variance, for a given method. It measures the dispersion of the systematic biases of the laboratories for a given state of the art method.

In ringtests, this value should be respected, provided that participants are skilled at using the method. Each participating laboratory should have its mean bias đ placed in the highest probability area, defined from $S_{đ}$, theoretical calculated distribution of the mean of the deviations.

Caution : $S_{đ}$ is different from Sd, which gives one of the limits of the acceptability area –

see figs 3 and 4 in page 4 of La Lettre de CECALAIT

So $S_{đ}$ takes into account both the Slab and the repeatability deviations. But, it weights the latter deviation by the number of samples in the trial and by the number of repetitions.

$S_{đ}$ is given by the following formula : $S_{đ} = \sqrt{S^2lab + S^2r/nq}$, with n : number of repetitions, q : number of samples

It is possible then to calculate the acceptability limit for the mean of the deviations to the true value (for each sample and for each participating laboratory) : **limit for đ**.

Lim đ = **limit for đ = ± 1.96 $S_{đ}$ ≈ ± 2 Slab**, in CECALAIT's ringtests.

Table 2, in page 2 of La Lettre de CECALAIT shows the results that were obtained.

For coliforms, Sr is much smaller than the present limit and đ is in good agreement with the present limit.

For staphylococci, if labs use the procedure : BP medium followed by confirmation using the coagulase test, none of the limits is respected. However, we already noticed (La Lettre de CECALAIT, n° 20) that most laboratories dramatically simplify this very cumbersome procedure, leading at a worse precision. Where the RPF method (the most used now) is used, the limits are respected.

For total flora, repeatability seems good. But, because of a high standard deviation of reproductiblity, the calculated limit for đ is markedly higher than the present limit. Maybe, in our ringtests, all participants are not as skilled and as involved as milk payment laboratories, which use this acceptability limit. Moreover, we are aware that participants may follow different standards (IDF and AFNOR), when, for instance, the agar temperature is different.

New acceptability limits for all microbial enumeration ringtests

From table 2, it seems that it is possible to set new acceptability limits for Sr and lim đ, the same for all enumeration methods studied here : microorganisms at 30°C, coliforms at 30°C – and also *E. coli*-, coagulase + staphylococci. The new acceptability limits proposed for these colony count methods are

Sr = 0.08 log and $S_{đ}$ = 0.15 log

which defines following limits limSr = **0.11 or 0.13 log** according to the number of samples and **limđ = ± 0.3 log**

Figures 1 and 2 (page 4 in La Lettre de CECALAIT) were taken from the analyses of individual repeatability in the statistical treatments of ringtests concerning the enumeration of coliforms in raw milk (Fig 1) and coagulase + staphylococci in cheese (Fig. 2). They show the effect of the new acceptability limit for the repeatability, using the parameter GRSL (see definition below fig. 2).

With the present limits for Sr, ie 0.14 log for coliforms and 0.25 log for staphylococci, GRSL = 59 % for coliforms and GRSL = 150 % for staphylococci. These limits are drawn in dotted vertical lines. With Sr = 0.08 log, the new acceptability limit proposed, GRSL is now 30 % for coliforms and 34 % for staphylococci. These new limits are drawn in bold vertical lines.

Figures 3 and 4 (page 4 in La Lettre de CECALAIT) were taken from the accuracy analyses in ringtests on the enumeration of staphylococci in cheese and microorganisms at 30°C in raw milk.

The present and the new acceptability limits are shown by rectangles of length 2 đ (the new ones in bold lines).

Figures 1 and 2 show clearly that these new limits fit rather well to what laboratories are able to perform. Anyway, they are not

penalized. The same conclusion can be drawn for staphylococci.

For the total flora, the new limit seems to correspond to a better separation between a large cluster of results and some rather abnormal results.

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In conclusion, these new harmonized acceptability limits should make it easier for laboratories to analyse their performances in ringtests. Nevertheless, it will remain necessary to check regularly the validity of these limits, especially if methods are improved