INSTRUCTION

CONCERNING THE REPORT OF A CECALAIT

MICROBIOLOGICAL QUANTITATIVE

PROFICIENCY TESTING

GENERAL PRINCIPLE OF THE DATA TREATMENT:

The general principle consists in an evaluation of the accuracy performances of the laboratory on the basis of the results obtained on the samples sent. **The statistical data treatment is carried out on the results in CFU / ml or g transformed into Log unit, to ensure a constant variance at different levels of the tested samples.**

The shaded parts are data for information only and do not fall within the criteria for evaluating the performance of the proficiency test

➢ The repeatability treatment contains the edition of the deviations between duplicates **in Log CFU / ml or g** per sample, and the calculation of the standard deviation of repeatability of the laboratory SL **(Table I).**

➢ The accuracy treatment is divided as follows:

- Calculation of the means of duplicates sent by the laboratory **in Log CFU / ml or g**.

- Calculation of the assigned values as follows:

1) Selection of the laboratories which have performed the tests before the deadline (defined for each proficiency test),
2) Selection of the laboratories according to the method used (optional according to the criteria),
3) Calculation, with selected laboratories, of an assigned value per sample with the algorythm A of the ISO 13528 standard (robust mean).

- Edition, per sample, of the means of duplicates, the calculated assigned values and the « laboratory mean-assigned value » deviation **in Log CFU / ml or g**. Calculation of the performance statistics in Log: the deviations mean ($\bar{d}$) and the standard deviation of deviations (Sd) **(Table II).** The assigned values are also presented in **CFU / ml or g** in the **table III**.

- Evaluation of the laboratory's global accuracy on a target of conformity (positioning of each laboratory according to the $\bar{d}$ and Sd calculated performance statistics), corresponding to the evaluation of the laboratory's performance **(Figure I).**

- For information, a z score calculation per sample **(Table IV)** and a graph of the values obtained **(Figure 2)** are realised.

A) TREATMENT OF THE LABORATORY'S REPEATABILITY (For your information):

1) Definition:

The repeatability measures the accordance between the successive results obtained with the same method and in the same conditions. They can be expressed, either by the standard deviation of repeatability, Sr, of the method (95% of the results are divided between $\pm$ 2.Sr around the rates mean), or by r, the absolute deviation between duplicates in 95% of cases (r = 2.77.Sr).

2) Presentation of the results: Table of the deviations between duplicates (Table I)

The first line of the table (N°) corresponds to the identification of the test samples.
The second line concerns **the deviations between duplicates per sample.**
The deviations between duplicates values must be compared to the absolute deviation between duplicates of the standardised method r mentioned below the table.
At the end of the line, you will find the standard deviation of repeatability of your laboratory, **SL,** and the number of the results taken into account for this calculation **NL**.

$SL = \sqrt{\frac{\sum W_i^2}{2n}}$; W_i : deviation between duplicates and n: number of samples

This value must be compared to the permissible absolute limit (Lim SL). Indeed, as the SL value is an estimation from n samples, a tolerance linked to the confidence interval of the calculation of SL (according to a low of χ^2 with an unilateral risk α = 5 %) and calculated from Sr (normative): **Lim SL**, is allowed. For 10 samples: Lim SL = 1.35.Sr.

Differences between duplicates per sample

SL = standard deviation of repeatability of the laboratory

Table I : (For your information) **REPEATABILITY - Table of differences between duplicates in log CFU/g**

N°	1	2	3	4	5	SL	NL
DIFFERENCES	0.189	0.041	0.000	0.109	0.192	0.093	10

Upper limits according to ISO 6888-2 / Amd. 1 : Differences between duplicates : r = 0.22 log Limit SL = 1.49 Sr = 0.12 log

DIFFERENCES : Differences between the laboratory's duplicates ** : missing value
SL : Standard deviation of repeatability per sample of the laboratory
NL : Number of determinations of the laboratory

B) TREATMENT OF THE LABORATORY'S ACCURACY:

1) Definition:

Accuracy measures the accordance between the true value and of the mean value that could be obtained after a large number of replications of the analytical procedure.

2) Presentation of the results:

2.1) ACCURACY table (Table II)

- The first line of the table (N°) corresponds to the identification of the test samples.
- The second line of the table **MOY** presents **the duplicates means in log CFU / ml or g** obtained by your laboratory. The results with an asterisk have been identified as abnormal according to the Grubbs test at 5%.
- The 3rd, 4th and 5th lines present **the assigned values of the test (REF), number of values retained and the standard deviation S* of determination of this value** per sample.
- The 6th line **DEVIATIONS (MOY – REF)** presents **the deviations of your laboratory's results in relation to the assigned values in log CFU / ml or g for each sample.** At the end of the line, you will find the performance statistics: **the mean $\bar{d}$ and the standard deviation Sd of the « mean of results – assigned value » deviations in log CFU / ml or g.**
  These calculated values are compared to the fixed limits (indicated below the table III), which are calculated, either from the repeatability (r) and reproducibility (R) values given in the standards or taken from the bibliography, or correspond to the limits already established (within the context of the control of interprofessional laboratories, for example).
  The value of the t Student test indicates the pertinence of any given judgement: if the value is higher than the threshold value (respectively 2.26 ; 2.57 and 2.78 for 10 ; 6 and 5 samples with n-1 degree of freedom), then the deviations mean of the laboratory is statistically different from 0 (at a threshold of 5 %), indicating a systematic analytical bias. This test enables also to conclude to systematic bias with a $\bar{d}$ value lower than the limit (tendancies).

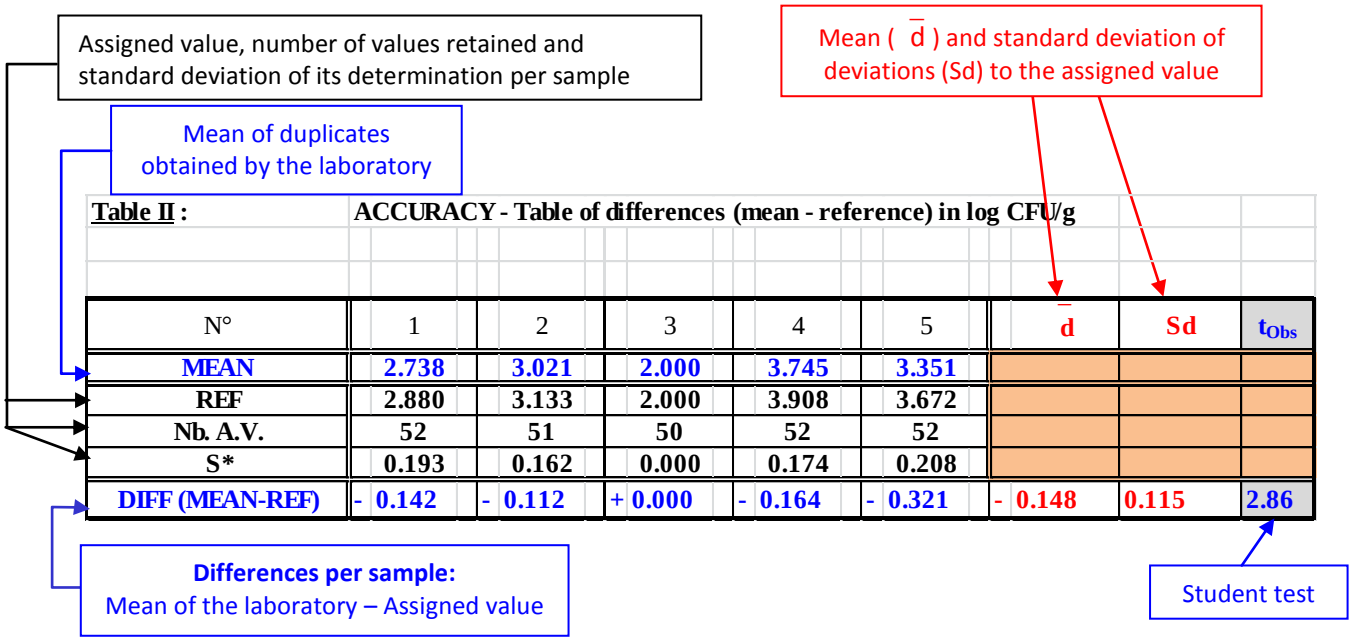


Table II : ACCURACY - Table of differences (mean - reference) in log CFU/g

N°	1	2	3	4	5	$\bar{d}$	Sd	t_{Obs}
MEAN	2.738	3.021	2.000	3.745	3.351			
REF	2.880	3.133	2.000	3.908	3.672			
Nb. A.V.	52	51	50	52	52			
S*	0.193	0.162	0.000	0.174	0.208			
DIFF (MEAN-REF)	- 0.142	- 0.112	+ 0.000	- 0.164	- 0.321	- 0.148	0.115	2.86

You will find below the accuracy table how the assigned values were calculated (number of laboratories, analytical method taken into account…).

2.2) Table of the assigned values in CFU / ml or g (Table III):

- The first line of the table (N°) corresponds to the identification of the test samples.
- The second line of the table corresponds to the **assigned values REF in CFU / ml or g (obtained by reverse transformation of the calculated results).**
  These values are supplied for information.

Table III : (For your information) **Table of references in CFU/g**

N°	1	2	3	4	5
REF	**759**	**1358**	**100**	**8100**	**4697**

Performance indicator

Individual performance :	For this evaluation, the goal has been reached	☺

A performance indicator informs you if the values of $\bar{d}$ and Sd obtained by the participant are within the tolerance specified by evaluation.

2.3) Table of z scores (Table IV) (For your information):

- The first line of the table (N°) corresponds to the identification of the test samples.
- The second line of the ***Z* SCORE** table presents the z score values per sample obtained by your laboratory. These values are calculated as follows:

$$z\,\text{Score} = \frac{(MOY - REF)}{S*}$$

The z score calculation enables an individual evaluation sample by sample of the performances of the laboratory in comparison with the global dispersion of the participating laboratories. This evaluation is strictly given for information and does not correspond to the evaluation of the laboratory's performance.

Table IV : (For your information) ***Z* SCORE - Table of *Z* SCORE values**

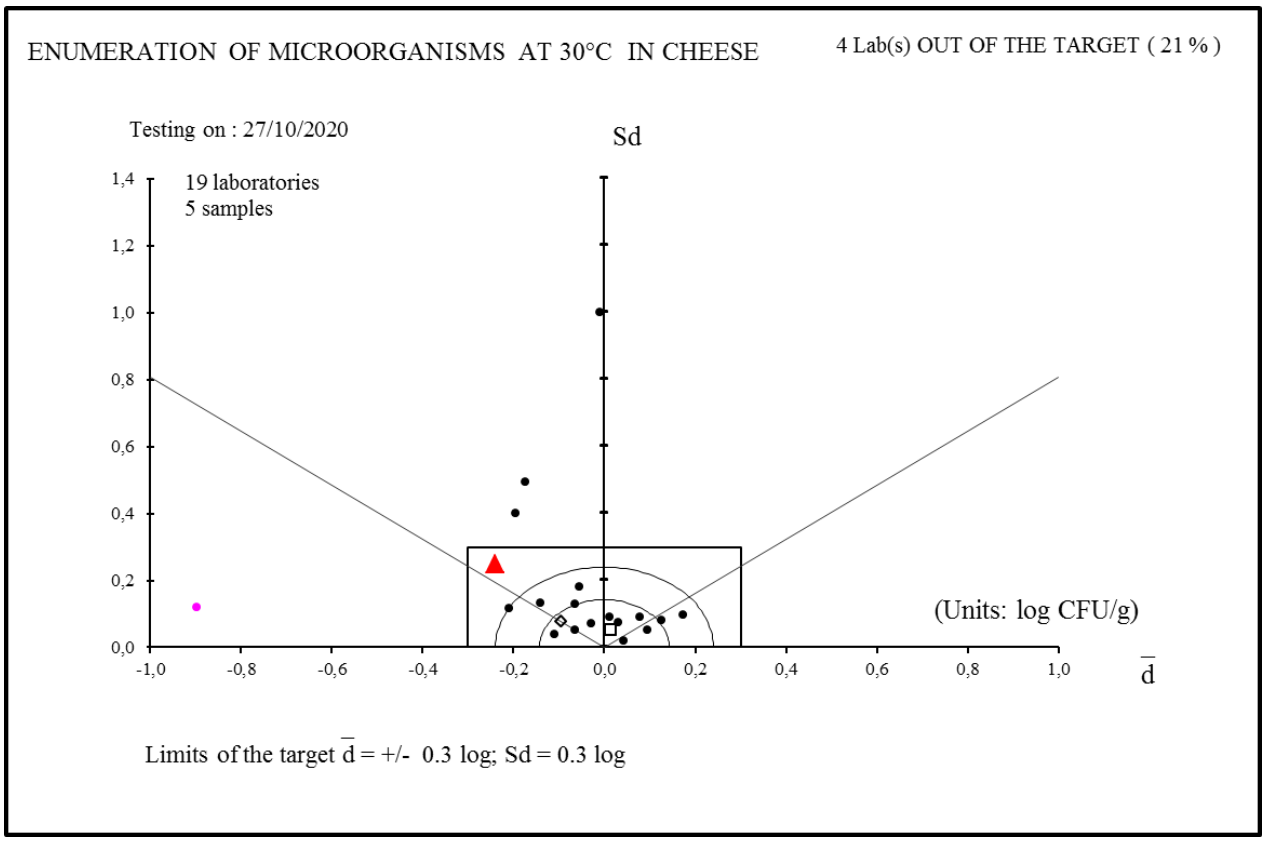
N°	1	2	3	4	5
Z SCORE	**- 0.74**	**- 0.69**	**N.C.**	**- 0.94**	**- 1.54**

Z SCORE = (MEAN - REF) / S* N.C. = z score value Not Calculated

2.4) <u>Personalised target of conformity (Figure 1 associated to table II)</u>:

The figure represents a general target of conformity, in which all the participating laboratories are represented from their $\bar{d}$ and Sd values obtained on the test. Your laboratory is represented on this graph by a **red triangle**. In this figure, $\bar{d}$ is on the X axis, and Sd is on the Y axis. A target (black rectangle) indicates the quality objective to be reached. Two symmetrical lines represent the significant limits of $\bar{d}$ (according to Sd) following the Student test at 5%: significant differences between the line and the X axis, and no significant differences between both lines.
In the case of different methods used by the laboratories, an identification is realised by the form and/or the colour of the marker.

This target corresponds to the evaluation of your laboratory's performance.



Positioning, as an additional laboratory, of the reference values of the groups of French and foreign laboratories. However, these data can only be provided if there are at least 5 French and 5 foreign laboratories.

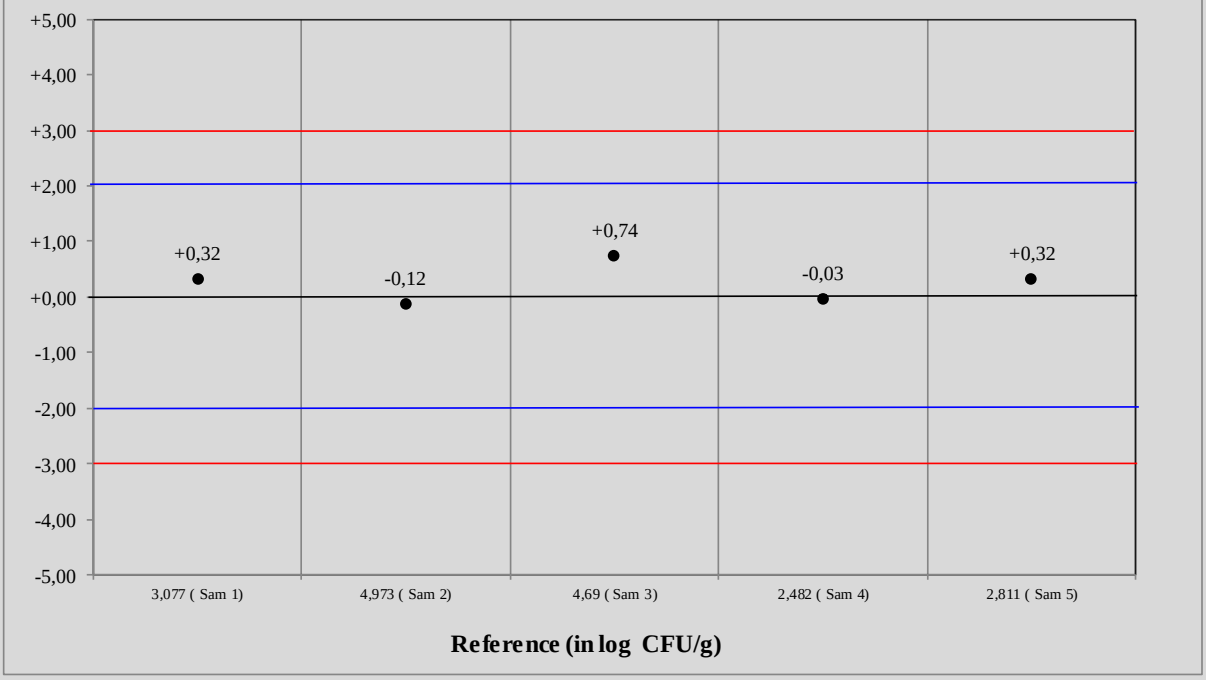
These two data are represented graphically on the compliance target by the following symbols:

☐ "robust average values" of the xx french laboratories (according to ISO 13 528)
◊ "robust average values" of the xx foreign laboratories (according to ISO 13 528)

2.5) z scores graph (figure 2 associated to table VI):

The figure represents a graphical positioning of the z scores obtained for the n samples of the test.
No relation between the scores obtained on each sample to evaluate the global performance of the laboratory is possible.
At best, this graph enables to visualise the positioning of each sample in relation to the statistical limits (± 2 and ± 3 corresponding to the alert and action limits) and to orientate the reflexion of the laboratory on its performance on one or many samples.

Figure 2 : (For your information) ACCURACY - Evaluation of the individual performances according to z score



$-2 \leq z \leq +2$: satisfactory $-3 < z < -2$ ou $+2 < z < +3$: warning signal $-3 \geq z \geq +3$: signal for action

C) EXPLOITATION OF THE PERFORMANCE STATISTICS:

1) Case of exceeding of the $\bar{d}$ tolerance limit

The deviations mean is an element indicating « the mean accuracy » of the participating laboratory.
First, it is necessary to observe the Sd value:

1.1) Low Sd value and t test value higher than the threshold value :

Case of a quasi constant deviation at all the levels indicating a systematic accuracy bias. In this case, the search of causes could be oriented towards a drift in the analytical procedure or to accuracy anomalies in the measurement.

1.2) <u>High Sd value and t test value lower than the threshold value</u> :

Case of a non systematic bias indicating a random deviation according to the samples. In this case, a more precise investigation may have to be carried out to refine the diagnostic:

- Search of outliers and recalculation of $\overline{d}$ and Sd without the sample(s) to do again the diagnostic at the first step.
- Influence of the measured analyte rate (level effect)
- Abnormally high repeatability on one or several samples (SL > Lim SL or deviation between duplicates > r).

2) <u>Case of exceeding of the Sd tolerance limit</u>:

Follow the procedure described §1.2.

Following the diagnostic of the « default » type met, a search will be carried out to identify the components of the method used (chosen process, material, reagents…), which can produce the problem in the considered test.
