SUMMARY OF LA LETTRE DE CECALAIT, N° 31 (4th quarter 1999)

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 reproducibility, 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 ringtests 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 $\bar{d}$ 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 ringtest, you can see that it allows to define two accuracy limits, symmetrical in relation to the zero difference. (see figures 3 et 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 quarterly 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 $\bar{d}$.

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 $\bar{d}$ placed in the highest probability area, defined from $S_{\bar{d}}$, theoretical calculated distribution of the mean of the deviations.

Caution : $S_{\bar{d}}$ 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 $S_{\bar{d}}$ 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.

$S_{\bar{d}}$ is given by the following formula : $S_{\bar{d}} = \sqrt{S^2lab + S^2r/nq}$, 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 $\bar{d}$.

Lim $\bar{d}$ = limit for $\bar{d}$ = $\pm 1.96\ S_{\bar{d}} \approx \pm 2\ S_{lab}$, 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 $\bar{d}$ 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 reproducibility, the calculated limit for $\bar{d}$ 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 $\bar{d}$, 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 $S_{\bar{d}}$ = 0.15 log

which defines following limits limSr = 0.11 or 0.13 log according to the number of samples and lim $\bar{d} = \pm 0.3$ 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 $\bar{d}$ (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.

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.

INTERESTING RECENT EEC REGULATION

➢ As usual, regulation n° 2377/90 of the Council concerning maximum residue limits of veterinary drugs in foods of animal origin has been amended.

Annexes I, II and III were amended by regulations 1931/1999 of 1999/9/9 (JOCE L 240 of 1999/9/10), 1942/1999 and 1943/1999 of 1999/.9/10 (JOCE L 241 of 1999/9/11), 2385/1999 of 1999/11/10 (JOCE L 288 of 1999/11/11) and 2393/1999 of 1999/11/11 (JOCE L 290 of 1999/11/12)

New residue limits were then inserted in the tables (annexes I and III). There were also other substances, not subject to MLRs, inserted in annex II.

Commission decision 1999/634/CE of 1999/9/9 amending decision 94/652/CE ...about **scientific cooperation among member states relative to foodstuffs**. (JOCE L 249 of 1999/9/22).

Council Regulation (EC) No 1602/1999 of 1999/7/19 amending Regulation No 2597/97, laying down **additional rules on the common organisation of the market** in milk and milk products for **drinking milk.** (JOCE L 189 of 1999/7/22).

Commission Directive 1999/71/EC of 1999/7/14 amending the Annexes to Council Directives 86/362/EEC, 86/363/EEC and 90/642/EEC on the fixing of **maximum levels for pesticide residues** in and on cereals, **foodstuffs of animal origin** and certain products of plant origin, including fruit and vegetables respectively (JOCE L 194 of 1999/7/27).

Commission Directive 1999/75/EC of 1999/7/22 amending Commission Directive 95/45/EC laying down specific purity criteria concerning **colours for use in foodstuffs** (JOCE L 206 of 1999/8/5).

Official Journals of the European Communities of the last 45 days may be consulted on Internet http://europa.eu.int/eur-lex
Older texts may be ordered on Internet http://www.eudor.com

FORTHCOMING EVENTS

➲ REMINDER

6 – 15 MARCH 2000 : LA ROCHE SUR FORON, FRANCE

Dairy Decade 2000

Europel
BP 141
74805 LA ROCHE SUR FORON cedex

tel : +33.4.50.03.01.03
fax : +33.4.50.03.10.64

13 – 16 MARCH 2000 BANFF, CANADA

Cheese ripening and technology

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http://www.fil-idf.org

➲ OTHER IDF EVENTS

3 – 7 APRIL 2000 SLOVENIA

Analytical week

IDF

13 – 14 APRIL 2000 NICOSIA, CYPRUS

Symposium on development strategy for the sheep and goat dairy sector

Mr G. Psathas
39, Dem. Severis Ave.
PO Box 22418
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CHYPRE

fax : +357/2.667313
e-mail : cmio@cytanet.com.cy

11 – 14 JUNE 2000 STRESA, ITALY

UDDER DEFENCES AND IMMUNOLOGY SEMINAR

IDF

➲ OTHER EVENTS

22-23 FEBRUARY 2000 PARIS, HOTEL SCRIBE

Seminar on Food Safety

Development Institute International
87, Bd Hausman
75008 PARIS

tel : +33 1.40.06.95.28
fax : +33 1.40.06.95.26

28-31 MARCH 2000 CNIT PARIS LA DEFENSE, FRANCE

4th Laboratory Forum

MCI
19, rue d'Athènes
75009 PARIS
FRANCE

tel : +33 (0)1.44.53.72.20°
fax : +33 (0)1.44.53.72.22
e-mail : mci-congres@wanadoo.fr

30 MARCH – 2 APRIL 2000 WADAHL HOTEL VINSTRA NORWAY

Milk protein Conference :
Structure and function
nutrition and health

Dpt of Food Science
PO 5036 NLH 1432 AAS

tel : +47 64.94.85.50
fax : +47 64.94.37.89

8-10 MAY 2000 **VELDHOVEN, NETHERLANDS** Euroresidue IV International Conference on veterinary drug residues in food	EuroResidue Foundation Dr L. A Van Ginkel c/o RIVM PO Box 1 NL-3720 BA BILTHOVEN THE NETHERLANDS	tel : +31 30 274 2613 fax : +31 30 274 4403 e-mail : euroresidue@rivm.nl Internet : http://www.rikilt.dlo.nl/euroresidue/
26-27 JUNE 2000 **NANTES, FRANCE** FOODSIM 2000 1st International Conference on simulation in food and bio industry	Society for Computer Simulation International	tel : +33 (0)2.51.78.54.54 fax : +33 (0)251.78.54.55 e-mail : ICSFBI@enitiaa.nantes.fr
28-30 JUNE 2000 **MELBOURNE, AUSTRALIA** Dairy Ingredients Science 2000	OzAccom Conference Services PO Box 164 Fortitude Valley QLD Australia 4006 *ou* DIAA National Office Kristine MANSER Level 6, 84 William Street Melbourne VIC Australia 3000	tel : +61 (0)7 3854 1611 fax : +61 (0)7 3854 1507 e-mail : ozaccom@ozaccom.com.au tel : +61 (0)3 9670 0422 fax : +61 (0)3 9642 8144 e-mail : diaa@dairy.com.au

AFNOR VALIDATION

➢AFNOR (French standardisation body) validated recently the following alternative methods :

- Transia Plate Salmonella SEP distributed by DIFFCHAMB, an ELISA test for the detection of all serotypes of mobile *Salmonella* in human or animal foods, also suitable for environmental tests.

- BAX *Salmonella* system, distributed by QUALICON TM.

➢ The following alternative methods were slightly amended, but are still validated, without any supplementary studies until respectively june 2000 and january 2002 : Probelia *Salmonella* sp. and Probelia *Listeria monocytogenes*.

➢ The lithium content of the RAPID'L MONO culture medium for the detection of *Listeria monocytogenes* was changed. After supplementary studies, the medium remains validated until september 2002

➢validation renewal

- The validation of Oxoid *Salmonella* rapid test (OSRT), distributed by OXOID was renewed for four years.

- The validation of the *Listeria* spp detection test kit, distributed by Transia is is about to be renewed

➢The following alternative methods are no longer validated: detection of biological molecules or microorganisms, Transia histamine tube

THE ROLE OF AFSSA AND LCHA AT THE NATIONAL INTERNATIONAL AND EUROPEAN COMMUNITIES LEVEL

(Abstract of the lecture given by Mr LALOUX of AFSSA at CECALAIT's annual general meeting)

In July 1998, a new french law relative to the reinforcement of public health surveillance and the control of the safety of the products and devices applied in human health created three public bodies :

- The National Institute for Public Health Surveillance (IVS)

- An Agency for Health Product safety (AFSSAPS)

- An Agency for Food Safety (AFSSA).

They depend on the Ministries of Health, Agriculture and Finance (Consumption and consumers)

THE MISSIONS AND STRUCTURE OF AFSSA

AFSSA has about 700 employees and its mission is to improve the National Survey System for food safety. It has three major tasks:

- For foods and feeds, to evaluate the health and nutrition risks, linked :
  - ✶ to the way they were produced, processed, preserved, stored or sold,
  - ✶ to animal diseases or to the use of veterinary drugs,
  - ✶ to the use of plant-care products, GMOs…
- To provide scientific and technical advice on animal health and well-being policy of the Ministry of Agriculture. The Agency also helps evaluate the consequences of this policy on food and feed safety,
- To issue market approvals for animal drugs

The Agency works together with five specialized Committees, among which, one is dealing with nutrition problems (CNERNA) and another with public health (CSHPF). It is divided into 4 Directions dealing with :

- veterinary drugs,
- animal health and well-being,
- evaluation of health and nutrition risks,
- food hygiene.

The former CNEVA (National Centre for Veterinary and Food Studies), with its many laboratories joined AFSSA in march 1999 . Among these, the LCHA (Laboratoire Central d'Hygiène Alimentaire - Central Laboratory for Food Hygiene) is concerned by all kinds of food.

THE ACTIVITIES OF LCHA

They are divided into three areas : research, development and technical support. For dairy products, the LCHA deals with :

- physico-chemical, organoleptic and hygienic quality monitoring,
- environmental contaminants evaluation and monitoring (pesticides, industrial pollutants, heavy metals, radionuclides)
- microbial enumerations,
- microbial contaminants (toxins).

For all these activities, LCHA works together with other national, European Communities or international organisations for standardisation, food safety, methods of analysis… for instance CEN (European Communities Standardisation Committee), Codex Alimentarius Committees...

The LCHA is the European Communities reference laboratory for food hygiene. So it serves as a liason between the 15 national reference laboratories (one for each European Community country) and local official laboratories.

In fact, the activities of the LCHA concern as well food hygiene and safety as control of international food trade.

INTERESTING NEW STANDARDS

IDF STANDARD

IDF 178A :1999. MILK AND HEATED MILK. Determination of acid soluble β-lactoglobulin content. Reverse phase HPLC method.

This standard, equivalent to ISO/CD 13875 is the final version of the 1996 provisional standard.

EUROPEAN STANDARD

European prestandard ENV 13005, august 1999 (ICS 03.120.30 and 17.020, ie « application of statistical methods» and « metrology and measurement in general »). Guide to the expression of uncertainty in measurement

DRAFT STANDARDS

ISO / DIS 1211, 1999. Milk. Determination of fat content. Gravimetric method (reference method)

ISO / DIS 13884, 1999 : Animal and vegetable fats and oils. Determination of isolated *trans* isomers by infrared spectrometry.

EN ISO / DIS 14891, 1999 : Milk and milk products. Determination of nitrogen content : routine method using combustion according to the Dumas principle

➢ We also noticed

➔ a set of european standards about materials and articles in contact with foodstuffs : ENV 13130 (7 parts) and EN 631-2

➔ standard EN 12830 about temperature recorders for frozen foods and ice cream.

LIST OF BIBLIOGRAPHIC REFERENCES

It is the list of references that we noticed in our litterature survey during the past months and that we decided to put into our data base on dairy analytical techniques. Should you be interested in any of these references, contact us, please.

NB : we remind you that we can copy neither book nor standard

.➢ We also noticed

• The World Dairy situation 1999, IDF Bulletin n° 339, 1999, 55 pages
Global survey of world production, transformation, trade and consumption of dairy products.

• An english glossary upon mastitis in IDF Bulletin, 1999, n° 338

• A special issue of International Dairy Journal, 1999, v. 9, N. 1, upon Recombinant dairy starters, probiotics and prebiotics : scientific, technological and regulatory challenges

• The acta of symposium Caseins and caseinates : structures, interactions, networks. Hannah Research Institute Symposium, 22-23 May 1997, in the same Journal, 1999, v. 9, N. 3/6

For further information, see www.elsevier.com/locate/iidairyj.