

SUMMARY OF LA LETTRE DE CECALAIT, N° 36 (1st quarter 2001)

(Translation : A. BAPTISTE, Correction : H. LAMPRELL)

AFNOR VALIDATION

AFNOR (French standardisation body) recently validated the following alternative methods : **LUMIPROBE 24H *Salmonella* sp.**, manufactured by Europrobe, validated on 2000/11/29 : a detection kit for *Salmonella*, applicable to eggs and egg products.

➤ Since 1991, AOAC International (Association of Official Analytical Chemists) have been developing a « Performance Tested Methods » program to provide an independent third-party review of test kit performance claims. Every year the Journal of

AOAC International presents the complete list of the test kits thus certified. The latest one is issued in **JAOAC, 2000, Vol. 83, N°5, pg 74A-76A**. It shows 35 test kits : 10 for detection of antibiotics, 20 for detection of pathogen (mostly *Salmonella* and *Listeria*) and 6 for the detection of various toxins or contaminants. Eight test kits are both AFNOR validated and AOAC certified :

- Oxoid Rapid Test, Transia Plate *Salmonella* Elisa, Dynabeads, BAX, TECRA and EIAFoss for the detection of *Salmonella* spp,
- Oxoid Rapid Test and Vidas for the detection of *Listeria* spp.

INTERESTING NEW STANDARDS

CEN STANDARDS

↪ **NF EN 12014-1/A1. February 2001 Foodstuffs - Determination of nitrate and/or nitrite content - Part 1 : general considerations**

↪ **In January 2001, a set of standards for the determination of some vitamins by high performance liquid chromatography in foodstuffs :**

- EN 12821 : Determination of Vitamin D. Measurement of cholecalciferol (D3) and ergocalciferol (D2).

- EN 12822 : Determination of vitamin E - Measurement of α , β , γ , and δ -tocopherols.

- NF EN 12823-1 & -2, Foodstuffs - Determination of vitamin A. Part 1 : measurement of all-trans-retinol and 13-cis-of β -carotene.

↪ **the new EN ISO 9000 collection of 3 standards (december 2000) Quality management and quality assurance standards : ISO 9000, 9001 & 9004**

News

➤ FDA (Food and Drug Administration) is thinking of changing the labelling of nutritional facts. The labelling of *trans* fatty acids would become compulsory and they should be summed up with saturated fatty acids. Further information at <http://www.fda.gov>

➤ In december 2000, a world convention on persistent organic pollutants (POPs) was held in Johannesburg. There, 122 countries agreed on banning or limiting the use of 12 POPs : pesticides, thermic fluidsincluding PCBs, dioxins and furans.

Results of the European Programme on *Clostridium perfringens*

Before accepting a standard as an European standard, CEN (*the EU standardization body*) requires precision data, which must have been validated by collaborative studies, as specified in standard ISO 5725. At the end of 1996, the European Community launched a 4 year project to validate six ISO microbiological methods for acceptance as standards. These are the methods of detection and/or enumeration of the following pathogens : *Bacillus cereus*, *Listeria monocytogenes*, coagulase positive *Staphylococcus*, *Clostridium perfringens*, *Salmonella*.

We have already reported on *Bacillus cereus* (see La Lettre de CECALAIT n° 26), on *Listeria monocytogenes* (see La Lettre de CECALAIT n° 30) and on coagulase positive staphylococci (see La Lettre de CECALAIT n° 35). Since then, the study on *Clostridium perfringens* is also finished. Its results and conclusions were shown during a meeting among the contractors in december 2000 and the final report will soon be issued.

Three contractors are involved in this project :

- ◆ AFSSA, France, also coordinator of the project,
- ◆ RIVM, Netherlands,
- ◆ MAFF-CSL, United Kingdom.

Each of them is in turn responsible for a study. RIVM was thus leader for the study on *C. perfringens*.

Among sub-contractors, CECALAIT was responsible for preparation, development, definition of preservation parameters and shipping of the cheese samples.

1) *CLOSTRIDIUM PERFRINGENS* : GENERAL POINTS AND ENUMERATION METHODS

It is an anaerobic, Gram positive, sporeforming rod, able to produce several food poisoning toxins during sporulation. It is considered as the 3rd cause of foodborne illnesses throughout the world (in THOLOZAN et al.).

It is widely distributed in the environment and frequently occurs in the intestines of humans and healthy animals. It often contaminates meat based food, either directly, or via the environment. Spores persist in soil, sediments and areas subject to fecal pollution. In favourable conditions : temperature between 15°C and 50°C, pH about 7, aw between 0.93 and 0.945, its generation time is very short. In the cold, some strains may still grow, but usually growing stops quickly : the number of vegetative cells is reduced, but spores survive. On the contrary, most spores are killed during cooking, but in some strains, they resist, allowing the microorganisms to multiply in prepared food, especially when it is cooled down too slowly.

Perfringens poisoning is caused by the consumption of food contaminated by quite a large number of vegetative cells (from 10⁵ to 10⁸/g of food, according to the literature!) : the cells multiply in the intestine and sporulate, releasing toxins. Symptoms : diarrhea and intense abdominal cramps, begin 6 to 48h after ingestion of contaminated food and are usually over within 24h. Most outbreaks of perfringens poisoning implicated meat, meat products or gravy.

REGULATIONS

Microbiological criteria for food seldom refer to *C. perfringens* itself, at least in the french regulations. For most foods (meat, pastries...), neighbouring criteria are specified, wider and not so well defined, eg sulfite-reducing *Clostridia* or sulfite-reducing anaerobes. In dairy products, these criteria only concern some milk-based products intended for particular nutritional use. When these products « were not heated in their bottles and might need a liquid adjunction before consumption », the criteria are :

In 1g of dry product or 10 g of liquid product :

- *C. perfringens* : absence,
- sulfite-reducing *Clostridia* at 46°C : < 10.

(French regulations : ministerial order of 1978/3/30, in note de service DGAI n° 2000-8155)

METHODS

→ ISO 7937 (1997) and EN 13401 (1999)

ISO 7937 (1997) is the horizontal reference method for the enumeration of *Clostridium perfringens*. CEN standard EN 13401 (1999) is practically the same, except for confirmation of presumptive *C. perfringens*. Indeed, it allows a choice between confirmation using lactose-sulfite medium (as in ISO 7937 (1997)) or using motily-nitrate & lactose-gelatine medium (as in ISO 7937 (1985)).

So both techniques have been included in this validation study in order to compare their performances and later to possibly harmonise the two standards.

Principle of standards ISO 7932 and EN 13401 is as follows, after preparation of the initial suspension.

- inoculate two sterile empty Petri dishes with the initial suspension or each of the serial dilutions,
- Pour egg-yolk free tryptose-sulfite-cycloserine agar (SC), maintained at 47°C and mix well,
- after solidification, add an overlayer of the same SC agar,
- incubate anaerobically at 37°C for 20h,
- count the black colonies of presumptive *C. perfringens*,
- confirm characteristic colonies (usually 5), retained for the enumeration.
 - In standards ISO 7937 and EN 13401, the confirmation method is based on high gas production and the presence of a black precipitate, in lactose-sulfite medium after anaerobic incubation at 46°C.
 - However standard EN 13401 describes an alternative confirmation method using the combination of two other tests which must be performed with the **same** well-separated characteristic colony :

- ◆ the first test uses the motility-nitrate reduction medium, where, after anaerobic incubation at 37°C, *C. perfringens* are non motile and reduce nitrate to nitrite, thus forming a strong red colour after adding a nitrite-detection reagent,
- ◆ The second test is based on high gas production and the presence of a yellow colour in lactose-gelatine medium, then on gelatine liquefaction.

The study was aimed at the determination of repeatability, *r*, and reproducibility, *R*, values for the method described in ISO 7937, but with each of the confirmation techniques.

2) COLLABORATIVE STUDY

As in the other studies of the project, the samples used were :

- reference material (capsules prepared by RIVM containing milk powder contaminated with spores of *Clostridium perfringens*),
- three different artificially contaminated food matrices :
 - ◆ raw milk cheese,
 - ◆ dried meat, prepared by MAFF-CSL,
 - ◆ dried animal feed, prepared by RIVM. But, due to the stickiness of this matrix, direct contamination was impossible. Therefore artificially contaminated milk powder was added to the feed.

They were all inoculated, at different inoculum levels, with spores of an appropriate *C. perfringens* strain*, and also with a simulated autochthonous flora for cheese and meat. For the feed however, the natural contamination flora was kept as background flora.

* originated from food or from patient material.

The final contamination levels are given in table 1, page 3 in La Lettre de CECALAIT n° 36.

Homogeneity and stability were checked before the beginning of the study.

The collaborative study took place in January and February 2000 and involved 17 laboratories from 13 European countries.

The analyses were made in blind duplicate and most laboratories tested all samples and performed both confirmation techniques.

3) RESULTS

Shipping and reception of the samples were generally found satisfactory.

Concerning the operating procedure, the incubation conditions in the lactose-sulfite confirmation medium varied between participating laboratories. Indeed the procedure described in ISO 7937 did not seem clear enough. Thus some laboratories incubated test tubes anaerobically, whereas others did not. Furthermore, these had difficulties with reading of the gas formation. However, these deviations were regarded as of minimal

influence on the final results and did not lead to any exclusion of results.

After log transformation and exclusion of outliers, using Duncan's Multiple Range test, repeatability and reproducibility were determined. As usual, for the calculation ISO 5725 was followed, but also standard project EN ISO 16140 (using the median value) which seems to fit better to microbiological methods.

Tables 2 and 3 in La Lettre de CECALAIT, pages 3 and 4 show the results obtained with the latter method. Nevertheless, the values obtained, using either method of calculation were almost the same.

Tables 2 and 3 show that repeatability and reproducibility vary somewhat among the different food types and the different contamination levels. As expected, the lower values were obtained with reference material. The higher ones were obtained for the dried animal feed at the lowest contamination level. These variations might be explained by the difference in the matrix, or by the different ways of samples contamination. Most of the time, the highest precision values were observed for the lowest contamination level. This was the case for cheese and feed, but was not so clear for meat.

However it was possible to calculate the average repeatability and reproducibility values as the arithmetic mean of the values obtained in the three levels (see table 4, page 4 in La Lettre de CECALAIT).

As in tables 2 and 3, table 4 also shows that the precision data obtained with either confirmation technique are very similar. As both techniques show equal performance, it seems desirable to leave the user the choice which one to perform.

4) CONCLUSION

The conclusions of this study of the reference methods for the enumeration of *Clostridium perfringens* found both ISO 7937 and EN 13401 methods satisfactory. Nevertheless the following recommendations to CEN and ISO were drawn up and will be presented at the next meeting for this programme in June 2001, in Bern :

- ◆ to include in both standards the precision data calculated in this study using project EN ISO 16140.
- ◆ to allow a choice in ISO 7937 between two techniques for confirmation of presumptive *C. perfringens* colonies : one, using lactose-sulfite medium, the other the combination of motility-nitrate and lactose-gelatine medium. This will lead to the harmonisation of standards ISO 7937 and EN 13401.
- ◆ to improve, in both texts, the description of the incubation conditions for lactose-sulfite medium confirmation test. It will be necessary to specify if anaerobic conditions are compulsory or optional.

The list of abbreviations and bibliographic references are in « La Lettre de CECALAIT »

INTERESTING RECENT EU REGULATION

EUROPEAN UNION

Commission Directives 2000/81/CE and 2000/82/CE of 18 and 20 December 2000 amending the Annexes to Council Directives.... 86/363/EEC....on the fixing of **maximum levels for pesticide residues** infoodstuffs of animal origin.....(JO L 326 of 2000/12/22 and JO L 3 of 2001/1/6).

Regulation 2908/2000 of 2000/12/29 amending annexes I and II of **regulation n° 2377/90** of the Council concerning **maximum residue limits of veterinary drugs in foods** of animal origin.(JOL 336 of 2000/12/30)

Commission Directive 2001/5/EC of 12 February 2001 amending Directive 95/2/EC concerning **food additives other than colours and sweeteners** (JO L 55 of 2001/2/24)

Commission Regulation 213/2001 of 9 January 2001 laying down detailed rules for the application of Council Regulation (EC) 1255/1999 as regards **methods for the analysis and quality evaluation of milk and milk products** and amending regulations (EC) No 2771/1999 and (EC) No 2799/1999 (JO L 37 of 2001/2/7)

This 99 page text aims at providing operators of the milk and milk products sector with a single text bringing together the reference and/or routine methods to be used when analyzing and evaluating the quality of their products and also establishing the scope and rules for applying those methods. This was specified before in at least 10 different regulations.

Thus, in this text will be found :

➔ First some general provisions :

- The list of reference methods applicable to milk and dairy product analyses. Under the common markets organization, **only** methods included in this list –updated every year- may be used. Most of them are published by international organizations.
- A procedure for checking results of routine methods against reference methods.
- A procedure for validation of reference methods by checking that they meet predetermined precision criteria concerning repeatability and reproducibility limits (calculation based on ISO 5725 standard, for chemical analyses). If a limit has been exceeded, analytical results may be evaluated following a method given in this text to determine the critical difference from that limit.
- An internal method of control of the quality of chemical analyses.
- A procedure to be used in case of disputes over the results of analysis.

- A reference method to be used for sensory evaluation of butter –for other dairy products, IDF 99C :1997 or equivalent should be used.

- A general procedure to check the performance of assessors and the reliability of results of sensory evaluation.

➔ seventeen descriptions of reference methods concerning :

- water /non fat solids / fat content of butter*

- tracers in butter, concentrated butter and cream*

- detection of cows' milk casein by isoelectric focusing *

- detection of coliforms in butter, skim milk, casein and caseinates, following a MPN method, based upon IDF 73A *

- lactose content of some dairy products.

- detection of rennet whey in skimmed milk powder (intended for public storage) by determination of glycomacropeptides by HPLC.

- detection of buttermilk in skimmed milk powder by the determination of phosphatidylserine and phosphatidylethanolamine by HPLC

- detection of antibiotic residues in skimmed milk powder.

- determination of skimmed milk content of compound feedstuffs.

- detection of starch.

- determination of moisture content of acid buttermilk powder.

- detection of foreign fats

** these methods are practically the same as in former repealed texts*

➔ details of amendments in regulations n° 2771/1999 and 2799/1999 and the list of repealed regulations. These are regulations n°s 1216/68, 3942/92, 86/94, 2721/95, 1080/96, 1081/96, 1082/96, 1854/96, 880/98 and 1459/98.

Regulation n°881/1999 which established the former list of reference methods to be used is then also repealed.

Commission regulation (CE) n° 466/2001 of 8 march 2001 setting **maximum levels for certain contaminants in foodstuffs** (JOCE L 77 of 2001/3/16).

Commission Directive 2001/22/EC of 8 March 2001 laying down the **sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and 3-MCPD in foodstuffs** (JOCE L 77 of 2001/3/16).

➤ also issued

Council Decision of 8 March 2001 supplementing Directive 90/219/EEC as regards to the criteria for establishing the safety, for human health and the environment, of types of **genetically modified micro-organisms** (JOCE L 73 of 2001/3/15)



➤ The final proposals for the new regulations concerning hygiene rules for food safety issued in december 2000. See : http://europa.eu.int/eur-lex/en/com/dat/2000/en_500PC438_01

➤ DG Health and Consumer Protection of the EU issued in february « Guidelines for quality control procedures of pesticide residue analysis in the EU » See :

http://europa.eu.int/comm/food/fs/ph_ps/pest/qualcontrol_en.pdf

➤ The new legislation on GMOs was adopted by the EU Council of Ministers on february 15th 2001. It will come into force 18 months after being issued in the Official Journal and will replace directive 90/220.

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 consulted following their topics on Internet <http://europa.eu.int/eur-lex/en/lif> or ordered on Internet <http://www.eudor.com>

Tables from Official French communications about analysers and methods authorized for milk payment purposes

Page 13 of la Lettre de CECALAIT, we show a table, given in a communication of the French Official Journal (2000/12/6) updating the list of the authorized analysers (in France) for milk payment purposes. These analysers were authorized after following the evaluation procedure described in La Lettre de CECALAIT, n°33 (2nd quarter 2000).

In the same way, page 14, we show a table, issued in another official communication (2000/12/8) about reference and routine methods authorized for analysing the milk for milk payment purposes.

Should you be interested in an english translation of these tables, please let us know by e-mail, fax or phone