SUMMARY OF LA LETTRE DE CECALAIT, N° 35 (4th quarter 2000)

Results of the European Programme on staphylococci

(Abstract of the lecture given by Mrs De Buyser at CECALAIT's Annual General Meeting 2000)

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) and on *Listeria monocytogenes* (see La Lettre de CECALAIT n° 30). Now the studies on staphylococci are also finished.

Three contractors are involved in this project :

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

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

Coagulase positive staphylococci, ie mostly *Staphylococcus aureus*, but also some other species, are pathogens, able to produce enterotoxins, responsible for food poisoning. Dairy regulation, eg EC directive 92/46 specifies that they should not exceed « m » = 100/ml, in raw milk meant for human consumption when brought on to the market ; in other dairy products (when marketed) they should be less than 10, 100 or 1000/ml or /g depending on the type of product.

The horizontal reference method for their enumeration is ISO 6888, which has 2 parts. Part 1 uses Baird-Parker agar, then a confirmation test by a coagulase reaction. Part 2 uses the same base medium; supplemented with rabbit plasma and bovine fibrinogen solution (RPFA).

The studies were aimed at the determination of repeatability,r, and reproducibility, R, values for each of these methods.

↳ COLLABORATIVE STUDIES

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

- reference material (capsules prepared by RIVM containing milk powder contaminated by *Staphylococcus aureus*),
- three different artificially contaminated food matrices :
  - raw milk cheese,
  - dried meat, prepared by MAFF-CSL,
  - dried food : whole egg powder, prepared by RIVM.

They were inoculated, at different inoculum levels, with one or several *S. aureus* strains, with coagulase negative staphylococci and also with a simulated autochthonous flora for cheese and meat.

The final contamination levels are given in table 1 in La Lettre de CECALAIT, page 8. Homogeneity and stability were checked before the beginning of the study.

The collaborative studies took place in june 1999 and involved 24 laboratories from 16 European countries. The analyses were made in blind duplicate and most laboratories performed both methods. Let us remind you of the principle of the methods :

- For ISO 6888-1 :
  - inoculation of the surface of two plates of selective solid Baird-Parker medium, with the initial suspension or serial dilutions,
  - aerobic incubation at 37°C and counting of typical and/or atypical colonies at 24 and 48h,
  - confirmation by coagulase test on some typical and/or atypical colonies.
- For ISO 6888-2 :
  - inoculation in the depth of two plates of selective RPFA agar, with the initial suspension or serial dilutions,
  - aerobic incubation at 37°C and counting of typical and/or atypical colonies at 24 and 48h,

↳ RESULTS

Shipping and reception of the samples were generally found satisfactory.

There were some discrepancies among laboratories concerning the differentiation between typical and atypical colonies on Baird-Parker medium.

After log transformation and exclusion of outliers, repeatability and reproducibility were determined. Calculation followed ISO 5725 as usual, but also standard project EN ISO 16140 (using the median value) which seems to fit better to microbiological methods.

The values obtained, using these two methods of calculation were almost the same. Tables 2 and 3 in La Lettre de CECALAIT, pages 8 and 9 show the results.

There are few differences between the different food matrices, except with cheese analysed with Baird-Parker medium. Repeatability and reproducibility are overall better with RPFA agar. Table 4, page 9 in La Lettre de CECALAIT shows the mean precision values obtained in the collaborative studies.

Let us remind you of the values obtained after grouping together the results of several CECALAIT proficiency studies (cf Lettre de CECALAIT, n°20) :

- r(log) = 0.52 and R (log) = 1.13 for Baird-Parker medium;
- r(log) = 0.19 and R(log) = 0.43 for RPFA medium.

They are higher than the values given above. But, we have to consider that :

- first, the participating laboratories were more diverse than those involved in the European project.
- then, a lot of participating laboratories hardly followed the standardized procedure, especially with Baird-Parker medium.

However, with RPFA on milk, the values observed in our studies were rather close to those observed with cheese in the European project. The precision values, calculated from the European studies do not seem out of reach of national or local laboratories.

↳ CONCLUSIONS

The conclusions of the European Project found both methods satisfactory and gave the following recommendations to CEN and ISO -they were all accepted in the meanwhile- :

- to amend the standards with the performance characteristics calculated in the study, following project ISO 16140 ,
- to give the same status to both parts of the standard
- to study again the description of typical, atypical and non-staphylococci colonies in ISO 6888-1.

In France, some organizations concerned by food hygiene suggested changes in the microbiological criteria for staphylococci in dairy products.

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