Microbiology Proficiency Testing Scheme on "Real" Meat Samples

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Billions of microbiological analyses are performed each year throughout the world. The high quality of these analyses, as well as the accreditation of the laboratories (standard ISO/IEC 17025[1]) require use of reference materials.

Reference materials are used to assess the performance of a laboratory, validate analytical methods, calibrate measuring devices, improve technical skills of technicians, calculate the uncertainty measurement of laboratory.

Reference materials are rare and are commonly in lyophilized form or as powder form.
Reference materials based on food matrix exist and correspond to realistic conditions but their homogeneity and stability are unsatisfactory.

STUDY

A study has been conducted to fix acceptability criteria for production of minced meat spiked with several bacterial species (*E. coli*, *S. enteritidis*, *S. aureus*, *L. monocytogenes*, *B. cereus* and *C. perfringens*).
These spiked food samples are characterized by testing homogeneity (with T_2 Cochran's test and ANOVA) and stability (with linear regression test).

CONCLUSION

Two types of "real" food matrices spiked with several types of bacteria have been developed.
Matrices with a quantitative determination and matrices with a qualitative determination.
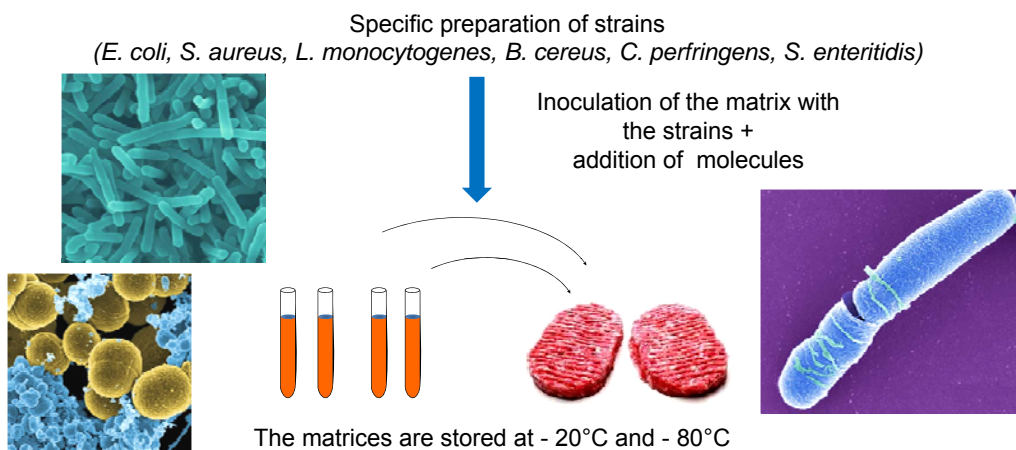Acceptability criteria has been fixed in order to produce these matrices.
The matrices present stability and homogeneity for 60 days at different storage temperatures:
- 20°C, - 80°C

PROSPECTS

These food samples have been used regularly in Bipea proficiency testing schemes since 2007. Every year, Bipea organizes at least three sets of proficiency testing programs on *E. coli* O157, on multiple parameters enumeration and on *Salmonella* and *Listeria*. Every set of proficiency testing schemes gathers at least 25 participants. This program is accredited by Cofrac.
Continuous research in the field should lead to the creation of new proficiency testing programs (*Vibrio cholerae*, *Pseudomonas*, *Campylobacter*, *Bronchothrix* and lactic acid bacteria). Bipea was also audited this summer to be accredited according to the ISO 17043 standard[3].

METHOD

Specific preparation of strains
(E. coli, S. aureus, L. monocytogenes, B. cereus, C. perfringens, S. enteritidis)

Inoculation of the matrix with the strains + addition of molecules

The matrices are stored at - 20°C and - 80°C

Acceptability criteria[2]

Quantitative sample

Homogeneity

Cochran's Test

$$T_2/(n-1) < 2$$

$$T_2 = \sum_{i=1}^{I} \frac{(Z_i - Z_+ / I)^2}{Z_+ / I}$$

z_i: enumeration of the sample *i*
z_+: sum of the enumeration of the *I* samples
I: sample total number

ANOVA

NS at 5%

Non checked criteria
$| X - Y | \le 0.3 \log_{10}$

X, Y: extreme value of series

Stability

Linear regression

Slope ≈ 0 (risk **at** 5%)

Non checked criteria
$| Y_0 - Y_n | \le 0.3 \log_{10}$

Y_0, Y_n: general means for T_0 et T_n

Strains	Days	Enumeration results in log						
		D0	D4	D7	D14	D30	D45	D60
E. Coli		3.78	3.67	3.62	3.67	3.58	3.69	4.21
B. Cereus		3.81	3.61	3.71	3.76	3.68	3.79	3.67
C. Perfringens		2.24	1.99	1.93	1.90	1.81	2.00	1.84
L. Monocytogenes		3.56	3.52	3.53	3.45	3.34	3.45	3.23
S. Aureus		3.67	3.70	3.73	3.48	3.21	3.71	3.47

Table 1. Example of bacterial enumeration for food matrix stored at – 20°C during 60 days

Qualitative sample

Samples are considered as homogeneous and stable if all their analysis results from Day$_0$ to Day$_{60}$ are positive

REFERENCES

[1]**ISO/IEC 17025** - General requirements for the competence of testing and calibration laboratories
[2]**ISO/IEC 13528** - Statistical methods for use in proficiency testing by interlaboratory comparisons
[3]**ISO/IEC 17043** - Conformity assessment – General requirements for proficiency testing

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