REPORT OF 2003/2004 ISO TRIALS ABOUT UNCERTAINTY MEASUREMENT

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I- INTRODUCTION

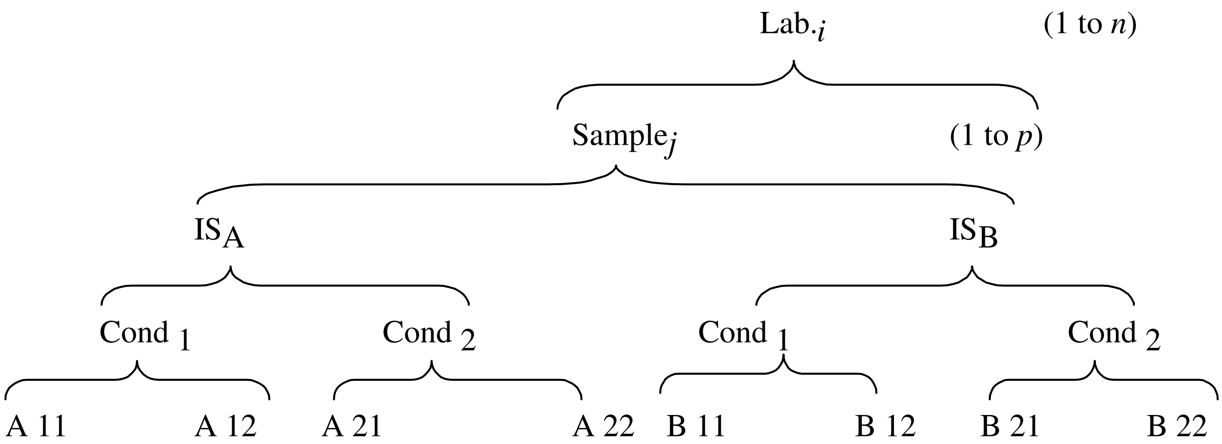
I.1- Objective

The objective of the trials was to estimate in different products (matrices) the components of the measurement uncertainty (MU) linked to taking the test portion (in the test sample) and to the preparation of the initial suspension.

The components linked to the sample storage and transportation are not taken into account since the trials are performed on different samples in each laboratory.

These trials were designed to calculate, in addition to the MU component strictly linked to the laboratory (from the initial suspension to the counting/confirmation of colonies and expression of results), the MU components linked to the initial steps of the analysis, from the test portion to the preparation of the initial suspension. It was supposed that this part of MU would not be (mainly) depending on the laboratory which estimates it, but rather on the type of matrix considered[1]. Thus we thought each laboratory would have been able to estimate its global MU, which would have comprised a common part, the MU on the test portion and the preparation of initial suspension (typical value defined per type of matrix), and an individual part, the MU based on the intra-laboratory standard-deviation[2].

I.2- Protocol



Where:

- 8 enumerations were performed on each sample j, analysed by a laboratory i.
- IS_A & IS_B stand for 2 initial suspensions prepared independently, as differently as possible (at least different operators, different balance, different diluent's batch,…).
- Cond.1 & Cond.2 stand for two groups of conditions differing as much as possible for the implementation of the analysis (different operators, different media batches, different incubators,…).
- The indexes $._1$ and $._2$ stand for two repetitions (that is two series of dilutions per initial suspension, for each type of conditions).

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[1] As a matter of fact, the contamination distribution of the target microorganiIS in the test sample, generally depends on the type of product considered, as well as on the interaction between the target microorganiIS and the matrix itself, and/or the annex flora.

[2] It is foreseen to detail this approach in the ISO/TS "*Microbiology of food and animal feeding stuffs – Guide on the measurement uncertainty of quantitative determinations*"

I.3- Participation

I.3.1- Participants

A total of **75 laboratories** participated in the collaborative trial. There were public control laboratories, private laboratories and agro-industry laboratories, from France (63/75), Croatia, Italy, Senegal, Indonesia, Singapore, China, Brazil, Chile.
A data file is the triplet {a laboratory, a flora, a food}. Each lab sent one to six data files, so that **118 data files** have been gathered.
Each file was supposed to contain 10 samples of precisely the same food. For each of the ten samples, there were supposed to be 8 exploitable enumeration results, with more than 10 counted colonies at the first dilution. Among the 118 collected data files, 27 data files had to be excluded because they were not consistent with these criteria. Partial results obtained for these **27 excluded data files** are presented in Annex II.
Exhaustive results concerning the **91 exploited data files** are presented in this report.

I.3.2- Microorganisms enumerated

Among the 91 exploited data files, 13 flora were enumerated : Aerobic mesophilic flora (35/91), Coliforms (16/91), E. coli Beta-glucuronidase positive (13/91), *Staphylococcus* coagulase positive (9/91), ASR, *Enterobacteriacea*, *Pseudomonas*, *B. cereus*, *Bifidobacterium, L. monocytogenes*, *Salmonella*, lactic flora, yeasts and moulds.
Three results were for the Aerobic mesophilic flora were obtained using the Spiral system.

I.3.3- Foods analysed

Among the 91 exploited data files, various foods were analysed :
- Meat (beef, veal, poultry, pork) and meat products (31/91): fresh meat, minced meat, sausages, sliced ham, pâté…- Dairy products (25/91): cheese, milk powder, milk, ice-cream
- Fruits and vegetables (15/91): dried figs, grated carrots, salad…
- Seafood (4/91): fish, shrimps
- Miscellaneous and composite foods (16/91): pastries, pet or cattle feeds, cooked snails…

I.4- Data interpretation

I.4.1- Definitions

Variance : mean of the deviation from the mean
Standard deviation : variance's root square, dispersion from the mean
Variance between conditions (s^2_{cond}): variance component linked to the variability between two conditions
Variance between initial suspensions (s^2_{IS}): variance component linked to the variability between two initial suspensions (and then mainly linked to the initial suspension heterogeneity)
Residual variance (s^2_{res}): last variance component, mainly linked to the variability between two repetitions = ***repeatability variance***
Total variance (s^2_{tot}): sum of the three variance components ($s^2_{tot} = s^2_{IS} + s^2_{cond} + s^2_{res}$) = ***intra-laboratory reproducibility variance***

I.4.2- Principles of the calculations

- plate-counts for both dilutions, we first calculated a weighted mean : the obtained result is then in cfu/ml or cfu/g
- Then the data are log-transformed (decimal logarithm): : the obtained result is then in log(cfu/ml) or log (cfu/g)
- The analysis of variance (ANOVA) is applied to each sample with the model :

result ~ condition + initial suspension

with random effects on the variables 'condition' and 'initial suspension'.

For each sample, the variance components are obtained :
- initial suspension variance component
- condition variance component
- residual variance

For each data files, the mean of the each variance is calculated, then the initial suspension standard deviation.

Note : samples excluded: samples with less than 10 colonies at the highest dilution
data files excluded: data files with less than 7 samples.

I.4.3- Detailed calculations

- Weighted mean : number/1.1 x $10^{dilution}$
- Log- transformation
- For each sample calculation of the sum of squared errors (SSE) and of the mean square errors (MS), sum of squared errors divided by the degrees of freedom

  - between conditions :

    $SSE_{cond} = 2(\text{mean}(cond_1 \text{ results}) - \text{mean}(cond_2 \text{ results}))^2$

    $MS_{cond} = SSE_{cond} / 1 = SSE_{cond}$

  - between initial suspensions

    $SSE_{IS} = 2(\text{mean}(IS_A \text{ results}) - \text{mean}(IS_B \text{ results}))^2$

    $MS_{IS} = SSE_{IS} / 1 = SSE_{IS}$

  - residual :

    $SSE_{res} = SSE_{tot} - SSE_{cond} - SSE_{IS}$

    $MS_{res} = SSE_{res} / 5$

- Variance component calculations :
  - $s^2_{IS} = \max \{0, 1/4 (MS_{IS} - MS_{res})\}$[3]
  - $s^2_{cond} = \max \{0, 1/4 (MS_{cond} - MS_{res})\}$

[3] Only positive variance components are kept

- For each data file :
    - mean (s^2_{IS})
    - mean (s^2_{cond})
    - mean (s^2_{res})

note : $s^2_{tot} = s^2_{IS} + s^2_{cond} + s^2_{res}$

- Calculation of the standard deviations : $s_{IS} = \sqrt{s^2_{IS}}$; $s_{cond} = \sqrt{s^2_{cond}}$; $s_{res} = \sqrt{s^2_{res}}$; $s_{tot} = \sqrt{s^2_{tot}}$

II-RESULTS

II.1- General results

II.1.1- Descriptive statistics

Descriptive statistics concerning the results obtained for 88 data files (without the Spiral enumeration results) are presented in the next table:

	Minimum	Median[4]	Maximum	Mean
s_{IS}	0.01 log	0.15 log	0.74 log	0.19 log
s_{cond}	0.01 log	0.07 log	0.58 log	0.10 log
$s_{rés}$	0.03 log	0.10 log	0.33 log	0.11 log
s_{tot}	0.04 log	0.23	0.78 log	0.26 log

Descriptive statistics for the 4 standard deviations[5]

The total standard deviation (s_{tot}), the square root of the total variance, cumulates the effects of all the sources of uncertainty, while the three "partial" standard deviations (s_{IS}, s_{cond} and s_{res}) represent each one a source of uncertainty: effects related to the heterogeneity of the food, and to the preparation of the initial suspension for s_{IS}, effects related to the operating conditions in the laboratory (especially with the personnel and the material) for s_{cond}, residual effects for s_{res}. These three "partial" standard deviations are the square roots of the variance components. It was thus awaited that s_{tot} is higher than the three others, which is checked well on the average, the median and the maximum in the table above. It is also observed that, among the three "partial" standard deviations, it is s_{IS} which reaches the highest values, with the highest average, median and maximum value. Even if this table does not make it possible to answer it directly, one can wonder right now what explains the weakest s_{IS} ("good" reproducibility) and on the contrary what explains the highest s_{IS} ("poor" reproducibility). According to the definitions, a Small total standard deviation can be obtained only thanks to 3 Small "partial" standard deviations (s_{IS}, s_{cond} and s_{res}). The effort must thus relate to all the sources of uncertainty simultaneously. On the other hand, as that will be illustrated in the tables hereafter, it is enough that one of the 3 standard deviations (in general s_{IS}) is very high so that s_{tot} is high.

[4]Median : central tendancy. Lecture key : among 88 s_{IS} (without spiral system) calculated, half is less than 0.15 (or equal to 0.15), the other half is more than 0.15 (or equal to 0.15).
[5] The 3 files with Spiral enumeration results were not take into account here.

II.1.2- The 10 highest s_{IS}

The table presents the 10 highest s_{IS}:

code	Food	Micro-organisms	s_{IS}	s_{tot}	s_{res}	s_{cond}
54	Dried figs	Aerobic mesophilic	**0,74**	0,75	0,07	0,01
11	Pâtés	Aerobic mesophilic	**0,72**	0,78	0,10	0,29
34	Fresh pork meat	Listeria monocytogenes	**0,70**	0,70	0,06	0,05
21	Cheese	Aerobic mesophilic	**0,59**	0,60	0,10	0,05
25	Pâté	Aerobic mesophilic	**0,46**	0,47	0,07	0,03
9	Raw milk cheese	*Escherichia coli*	**0,45**	0,47	0,10	0,06
9	Raw milk cheese	*Staphylococcus*	**0,43**	0,45	0,10	0,05
10	Salad	Coliforms	**0,41**	0,78	0,33	0,58
4	Fish	Aerobic mesophilic	**0,37**	0,51	0,29	0,20
2	Fish	Aerobic mesophilic	**0,36**	0,43	0,23	0,06

The 10 highest s_{IS}

A strong standard deviation s_{IS} (between initial suspensions) can be explained by a strong heterogeneity of the distribution of the micro-organisms in the matrix (examples: pies, cheeses...), and/or by a flora difficult to count, for example because it develops in a very irregular way (case of yeasts and moulds in dried figs).
In addition, it is observed (see tables detailed hereafter), that the highest s_{tot} are especially due to high s_{IS}.

II.1.3- Food categories and s_{IS}

Preliminary trials at the French level (see appendix II) had led us to suppose that s_{IS} was specific of a couple (matrix, flora), or even of a matrix, but independent of the laboratory. However, on the basis of tables detailed below, this assumption seems questionable. Indeed, when similar products were analysed by two different laboratories (see cheeses, pastry makings... in the tables detailed hereafter), differences do not appear totally negligible.

We tried to gain information, by gathering the matrices. A first classification (see I.3.3) did not appear relevant (no significant differences between meats, dairy products, vegetables, seafood, and miscellaneous/composite foods).
A second classification, based on the physical characteristics, made it possible to obtain more interesting results. Four categories were created on the basis of physical criteria:

i. liquids and powders: milk, coconut milk, dried milk, dehydrated onion powder , caseinate (9/91)
ii. minced / chopped solids and fluids: minced meat, mechanically separated meat, sausage meat, whipped cream, dairy ice cream, soya cream (16/91)
iii. (very) small solids: dehydrated parsley/mushrooms, grated carrots/celeriac, salad, shrimps, cereals, feeding stuffs, chopped hazel nuts (15/91)
iv. other solids: meat, cheeses, pastry... (51/91)

category	minimum	median	maximum	mean
i	0.02 log	0.08 log	0.17 log	0.08 log
ii	0.01 log	0.09 log	0.17 log	0.09 log
iii	0.08 log	0.17 log	0.41 log	0.19 log
iv	0.05 log	0.20 log	0.74 log	0.25 log

s_{IS} by category

To test if the factor category had a significant effect, an ANOVA was carried out. All the assumptions necessary to a parametric variance analysis not being joined together, it was necessary to use a nonparametric variance analysis (Kruskall-Walis). This Kruskall-Walis analysis is very significant ($p=5.10^{-7}$), which means that the factor 'category' is very significant, therefore that there is at least a category from which the s_{IS} are significantly different from the s_{IS} of the other categories. Pair comparisons, again by nonparametric tests (Mann-Whitney-Wilcoxon), made it possible to conclude that there is a significant difference between the first two categories on the one hand, and the two last categories on the other hand.

The two first categories have relatively low standard deviations s_{IS}. **For these categories, one can retain that the standard deviation is of about 0.1 log, whatever the laboratory and the flora.**

The third category corresponds to solids, made up of Small elements. One can to suppose that there is a spontaneous homogenisation of these products before the preparation of the initial suspension (and thus a more homogeneous distribution of the contamination) and/or that it is easier for the operator to manipulate these products. This third category corresponds to intermediate standard deviations: higher than those of the two preceding classes but without values as high as in the last category (other solid foods). Indeed, this fourth category corresponds to solid foods and includes very high s_{IS} (max 0.74 log). The standard deviations vary much (min 0.05 log, max 0.74 log) because this class is very heterogeneous by definition. At the interior of this fourth category, it was not possible to make relevant regroupings. **For the two last categories, it does not seem possible to give an order of magnitude independent of the flora and laboratory.**

II.1.4- Flora and s_{IS}

Except yeasts and moulds, which might have relatively high s_{IS}, we did not see any clear effect of the factor 'flora'. Anyway, results are insufficient to conclude, regarding this question.

II.2- Detailed results

Exhaustive results concerning the 91 exploited data files are presented below, per flora. A laboratory code is sent to each participant by electronic mail and used in the tables.
Appendix I provides basic examples of how to use these standard deviations. Appendix II provides partial results concerning the 27 excluded data files. Appendix III provides results concerning a former preliminary trial.

II.2.1- Aerobic mesophilic flora.

code	Food	category	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}
2	fish	iv	**0,36**	0,43	0,23	0,06
2	frozen minced veal meat	iv	**0,07**	0,25	0,24	0,06
3	pastries	iv	**0,12**	0,18	0,11	0,07
4	fish	iv	**0,37**	0,51	0,29	0,20
7	ready-to-eat cooked meals	iv	**0,24**	0,33	0,17	0,13
8	vacuum packed minced beef meat	ii	**0,09**	0,15	0,10	0,06
10	packed green salad	iii	**0,10**	0,45	0,17	0,41
10	dehydrated onion powder	ii	**0,17**	0,24	0,13	0,11
11	pâté	iv	**0,72**	0,78	0,10	0,29
11	pastries	iv	**0,05**	0,19	0,12	0,13
11	dehydrated mushrooms	ii	**0,14**	0,26	0,15	0,16
12	chicken neck skin	iv	**0,19**	0,20	0,06	0,02
13	cooked snails	iv	**0,06**	0,13	0,10	0,05
14	pastries	iv	**0,32**	0,35	0,11	0,08
20	chicken neck skin	iv	**0,14**	0,16	0,05	0,06
20	mechanically separated turkey meat	ii	**0,10**	0,13	0,06	0,05
20	mechanically separated chicken meat	ii	**0,10**	0,14	0,09	0,05
25	pâté	iv	**0,46**	0,47	0,07	0,03
26	raw milk cheese	iv	**0,16**	0,26	0,09	0,19
27	sliced ham	iv	**0,30**	0,31	0,06	0,05
30	pastries	iv	**0,09**	0,12	0,06	0,05
31	grated carrots	iii	**0,09**	0,14	0,08	0,08
32	fresh pork sausages	iv	**0,20**	0,24	0,12	0,05
34	fresh pork meat	iv	**0,70**	0,70	0,06	0,05
38	vanilla ice cream	ii	**0,03**	0,10	0,09	0,02
41	milk powder (environment)	i	**0,05**	0,14	0,10	0,08
42	milk powder	i	**0,02**	0,05	0,04	0,02
43	frozen shrimps	iii	**0,19**	0,20	0,05	0,05
44	frozen shrimps	iii	**0,09**	0,18	0,14	0,08
48	milk	i	**0,04**	0,12	0,06	0,09
49	corn starch	i	**0,09**	0,14	0,06	0,08
55	packed green salad	iii	**0,15**	0,20	0,06	0,11
72	caseinate	i	**0,03**	0,09	0,08	0,04
With spiral system :						
1	raw milk cheese	iv	**0,29**	0,38	*0,12*	*0,21*
24	dry feed for dog	iii	**0,18**	0,24	*0,13*	*0,09*
59	minced meat	ii	**0,17**	0,23	*0,15*	*0,05*

Standard deviations with Aerobic mesophilic flora.

II.2.2- Coliforms

code	Food	category	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}
1	vacuum packed minced beef meat	iv	**0,32**	0,35	0,11	0,07
3	pastries	iv	**0,16**	0,23	0,15	0,07
6	fresh beef meat	iv	**0,33**	0,35	0,05	0,09
10	packed green salad	iii	**0,41**	0,78	0,33	0,58
12	chicken neck skin	iv	**0,15**	0,20	0,12	0,06
20	chicken neck skin	iv	**0,07**	0,12	0,09	0,05
26	raw milk cheese	iv	**0,30**	0,33	0,09	0,10
29	mechanically separated chicken meat	ii	**0,10**	0,15	0,07	0,08
30	pastries	iv	**0,15**	0,19	0,09	0,07
32	fresh pork sausages	iv	**0,15**	0,31	0,23	0,14
44	raw milk cheese	iv	**0,11**	0,21	0,10	0,14
45	fresh meat	iv	**0,17**	0,22	0,10	0,09
58	frozen coconut milk	i	**0,12**	0,18	0,11	0,08
73	raw milk cheese	iv	**0,32**	0,48	0,28	0,03
74	whipped cream	ii	**0,07**	0,20	0,13	0,13

Standard deviations with Coliforms

II.2.3- *E.coli*

code	Food	category	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}
9	raw milk cheese	iv	**0,45**	0,47	0,10	0,06
16	raw milk cheese	iv	**0,09**	0,13	0,07	0,07
17	poultry meat (without skin)	iv	**0,27**	0,35	0,10	0,20
18	raw milk cheese	iv	**0,25**	0,27	0,07	0,06
19	poultry liver	iv	**0,12**	0,16	0,09	0,05
35	raw milk cheese	iv	**0,13**	0,18	0,12	0,03
37	frozen minced beef meat	ii	**0,13**	0,17	0,10	0,05
47	Soya cream	ii	**0,13**	0,44	0,15	0,39
50	raw milk cheese	iv	**0,29**	0,30	0,04	0,02
50	raw milk cheese	iv	**0,24**	0,26	0,08	0,05
51	raw milk cheese	iv	**0,13**	0,15	0,07	0,02
52	sausage meat	ii	**0,08**	0,11	0,07	0,03
59	minced meat	ii	**0,15**	0,19	0,08	0,09

***Standard deviations with* E.coli**

II.2.4- *Staphylococcus*

code	Food	category	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}
1	raw milk cheese	**iv**	**0,26**	0,33	0,16	0,14
16	raw milk cheese	**iv**	**0,08**	0,16	0,11	0,09
28	raw milk cheese	**iv**	**0,15**	0,24	0,17	0,08
46	Dry noodles	**iii**	**0,09**	0,13	0,08	0,05
50	raw milk cheese	**iv**	**0,15**	0,16	0,05	0,01
50	raw milk cheese	**iv**	**0,12**	0,14	0,05	0,04
9	raw milk cheese	**iv**	**0,43**	0,45	0,10	0,05
36	raw milk cheese	**iv**	**0,21**	0,22	0,06	0,04
71	raw milk cheese	**iv**	**0,20**	0,23	0,09	0,04

***Standard deviations with* Staphylococcus**

II.2.5- Other flora

code	Food	Micro-organisms	category	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}
10	dehydrated onion powder	yeasts+moulds	**i**	**0,08**	0,23	0,09	0,20
11	dehydrated mushrooms	Bacillus cereus	**iii**	**0,21**	0,26	0,12	0,09
15	poultry meat (without skin)	Pseudomonas	**iv**	**0,20**	0,34	0,14	0,24
20	mechanically separated turkey meat	ASR	**ii**	**0,05**	0,10	0,08	0,03
20	mechanically separated chicken meat	ASR	**ii**	**0,09**	0,14	0,09	0,06
21	raw milk cheese	Listeria monocytogenes	**iv**	**0,59**	0,60	0,10	0,05
22	cattle feeding powder	Enterobacteriaceae	**iii**	**0,31**	0,33	0,11	0,05
33	grated celeriac	Lactic flora	**iii**	**0,08**	0,25	0,14	0,20
39	milk powder	Bifidobacterium	**i**	**0,09**	0,14	0,08	0,08
40	dry parsley	Bacillus cereus	**iii**	**0,17**	0,27	0,18	0,12
45	fresh meat	Salmonella	**iv**	**0,21**	0,24	0,09	0,07
53	minced turkey meat	ASR	**ii**	**0,07**	0,25	0,12	0,21
54	dried figs	yeasts+moulds	**iv**	**0,74**	0,75	0,07	0,01
55	corn flakes	Moulds	**iii**	**0,32**	0,36	0,11	0,12
56	fresh chicken meat	*Enterobacteriacea*	**iv**	**0,35**	0,52	0,29	0,27
57	minced beef meat	*Enterobacteriacea*	**ii**	**0,01**	0,04	0,03	0,02
65	minced turkey meat	*Pseudomonas*	**iv**	**0,20**	0,34	0,14	0,24
70	hazel nuts	yeasts+moulds	**iii**	**0,28**	0,29	0,03	0,07

Standard deviations with the other flora

III- CONCLUSION

These trials aimed at characterizing the part of measurement uncertainty, in the case of microbiological enumerations, related on preparation of the initial suspension, they involved 75 laboratories from 9 countries.
Our primary objective was not reached as it was not possible to associate one typical value per food type. Indeed, this part of uncertainty might be dependent of the laboratory which determines it. The results of these tests were presented with members of ISO/TC34/SC9 at its last meeting (Parma, April 20-22, 2004). The SC9 deduced it was not possible to break up the measurement uncertainty. Only a global solution could be adopted.
On the other hand a publication of the results in a scientific journal was strongly recommended.

APPENDIX I : HOW TO USE STANDARD DEVIATIONS (INDICATIVE EXAMPLES).

As indicative examples, various calculations are presented on the basis of the results of one lab (code 20).

This laboratory sent 6 data files: 4 with food of category ii. and 2 others of category iv. Standard deviations are as follows:

code	food	category	Micro-organiIS	s_{IS}	s_{tot}	s_{res}	s_{cond}
20	mechanically separated turkey meat	ii	Aerobic mesophilic	0,10	**0,13**	0,06	0,05
20	mechanically separated turkey meat	ii	ASR	0,05	**0,10**	0,08	0,03
20	mechanically separated chicken meat	ii	Aerobic mesophilc	0,10	**0,14**	0,09	0,05
20	mechanically separated chicken meat	ii	ASR	0,09	**0,14**	0,09	0,06
20	chicken neck skin	iv	Aerobic mesophilic	0,14	0,16	0,05	0,06
20	chicken neck skin	iv	Coliforms	0,07	0,12	0,09	0,05

1st example simple use of s_{tot} for the same lab, same food, same flora:
Assumption : none. s_{tot} is calculated for 1 lab, 1 food, 1 flora.
For example, s_{tot} = 0.13 log for lab = 20, food = mechanically separated turkey meat and flora = Aerobic mesophilic.
If this lab has to express the uncertainty associated to an enumeration result R = 3 log (= 10^3 ufc/g = 1000 ufc/g), obtained by enumerating the aerobic mesophilic flora in mechanically separated turkey meat, then the 95% confidence interval around R would be :
R ± 2 s_{tot} = 3 ± 0.26 = [2.74 log; 3.26 log] = [$10^{2.74}$ ufc/g ; $10^{3.26}$ ufc/g]= [550 ufc/g ; 1800 ufc/g]

2nd example (use of s_{tot} for the same lab, similar foods, any flora):
Assumption : s_{tot} is the same for 1 lab, similar foods (in this case, mechanically separated meat), any flora (aerobic mesophilic or ASR)
Total standard deviation s_{tot} is obtained by taking the square root of the 4 variances s^2_{tot} for these two food products and these two flora :

$$s_{tot} = \sqrt{\frac{0.13^2 + 0.10^2 + 0.14^2 + 0.14^2}{4}} = 0.13$$

If this lab has to express the uncertainty associated to an enumeration result R = 3 log (= 10^3 ufc/g = 1000 ufc/g), obtained by enumerating any flora in mechanically separated meat, then the 95% confidence interval around R would be :
R ± 2 s_{tot} = 3 ± 0.26 = [2.74 log; 3.26 log] = [$10^{2.74}$ ufc/g ; $10^{3.26}$ ufc/g]= [550 ufc/g ; 1800 ufc/g]

3rd example (use of s_{tot} for the same lab, all foods of cat. i and ii, any flora):
Assumption : s_{tot} is the same for 1 lab, any food of cat. i and ii, any flora
Total standard deviation s_{tot} is obtained by taking the square root of the 4 variances s^2_{tot} of the food from category ii. :

$$s_{tot} = \sqrt{\frac{0.13^2 + 0.10^2 + 0.14^2 + 0.14^2}{4}} = 0.13$$

Same calculations as above but the confidence interval could be applied to any result R obtained by enumerating any flora in any liquid, or powder, or mixed/minced food.

4th example (common use of s_{res} and s_{cond} for the same lab, any food, any flora):
Assumption 1: s_{res} and s_{cond} are the same for one lab = 20, whatever the food and whatever the flora.
Assumption 2: s_{IS} ~ 0.1, for a food of category i or ii, whatever the flora.

From the standard deviations s_{res} and s_{cond} of this laboratory, the total standard deviation s_{tot} could be estimated by accepting that s_{IS}=0.1 for a category i. or ii. food.

- Calculation of the residual variance = mean of the s^2_{res} on the 6 data files :

$$s^2_{res} = \frac{0.06^2 + 0.08^2 + 0.09^2 + 0.09^2 + 0.05^2 + 0.09^2}{6} = 0.0061$$

- Calculation of the intercondition variance = mean of the s^2_{cond} on the 6 data files :

$$s^2_{cond} = \frac{0.05^2 + 0.03^2 + 0.05^2 + 0.06^2 + 0.06^2 + 0.05^2}{6} = 0.0026$$

- Calculation of the total standard deviation $\tilde{s}_{tot}$ with s_{IS}=0.1.

$$\tilde{s}_{tot} = \sqrt{0.0061 + 0.0026 + 0.1^2} = 0.14$$

If this lab has to express the uncertainty associated to an enumeration result R = 3 log (= 10^3 ufc/g = 1000 ufc/g), obtained by enumerating any flora in any food of cat. i or ii, then the 95% confidence interval around R is :
R ± 2 s_{tot} = 3 ± 0.28 = [2.72 log; 3.28 log] = [$10^{2.72}$ ufc/g ; $10^{3.28}$ ufc/g]= [520 ufc/g ; 1900 ufc/g]

APPENDIX II: EXCLUDED FILES

Among the 27 excluded files, only one was considered totally not computable (lab. 14, coliforms in pastries). For other files, approximate results are given below.

code	Food	Reason for the exclusion	Flora	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}	$s_{rés+cond}$ [6]	$s_{cond+IS}$ [7]
5	egg powder	too many low counts	ASR46°C	0.16	0.21	0.08	0.10		
10	onion powder	too many low counts	coliforms	0.58	0.62	0.17	0.16		
23	various foods[8]	too many different matrices	aerobic mesophilic	0.02	0.03	0.03	0.01		
34	fresh pork meat	protocol	aerobic mesophilic		0.20	0.06			0.19
34	delicatessen	protocol	aerobic mesophilic		0.13	0.06			0.11
35	cheese	too many low counts	*Staphylococcus*	0.21	0.44	0.39	0.00		
42	milk powder	too many low counts	spores	0.01	0.04	0.03	0.03		
60	pastries (éclairs)	too many low counts	coliforms	0.13	0.20	0.15	0.03		
60	sausages and sausage meat	too many low counts	*Escherichia coli*	0.27	0.30	0.09	0.10		
61	various delicatessen	protocol	aerobic mesophilic		0.48	0.05			0.48
61	various delicatessen	protocol	coliforms		0.43	0.19			0.39
62	fruit juices	protocol	yeasts moulds		0.05			0.05	
63	shellfish	protocol	*Escherichia coli* (NPP)		0.11	0.11			
64	various foods	too many different matrices	aerobic mesophilic	0.13	0.25	0.15	0.14		
64	various foods	too many different matrices	aerobic mesophilic	0.20	0.46	0.16	0.38		
64	various foods	too many different matrices	lactic flora	0.18	0.23	0.11	0.09		
64	various foods	too many different matrices	lactic flora	0.16	0.65	0.16	0.61		
64	various foods	too many different matrices	yeasts moulds	0.19	0.24	0.14	0.06		
64	various foods	too many different matrices	yeasts moulds	0.15	0.76	0.18	0.72		
66	various dairy products[9]	too many different matrices	*Streptococcus*	0.11	0.17	0.09	0.09		
67	semolina	protocol	aerobic mesophilic		0.13	0.08			0.10
67	semolina	protocol	moulds		0.08	0.07			0.04
67	semolina	protocol	coliforms		0.23	0.21			0.09
68	cheese	too many low counts	*Staphylococcus*	0.18	0.47	0.43	0.09		
69	white pudding	protocol	coliforms		0.61	0.11			0.60
75	cheese	too many low counts	*Enterobacteriacae*	0.64	0.66	0.18	0.05		

[6] This notation was used in case of inadequate protocol.
[7] This notation was used in case of inadequate protocol.
[8] Most of these foods were mixed (cat. ii).
[9] Most of these foods were liquid, powders, or fluids (cat. i or ii).
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APPENDIX III : FRENCH PRELIMINARY TRIALS

Preliminary trials had been carried out to evaluate the international trials feasibility. Results are presented next page. The principle was similar, but the number of samples was Smaller (maximum 5 for sausage meat, maximum 10 for other matrices). In some cases, the protocol was simplified (no s_{cond}).
Whatever the method and the laboratory, the s_{IS} of the common matrix, sausage meat, (bold figures) are close to 0.1 log, which is expected for category ii (cf. II.1.3) .
Lab 2' had intentionally homogenized the sausage meat before analysis, and the s_{IS} are even weaker. This same lab. had intentionally taken distant test portions in the pork meat product, so that the s_{IS} are very high.
Raw milk, a liquid (category i) is associated to the Smallest s_{IS}, whereas poultry legs and pork meat product, both in category. iv, have the highest s_{IS}.

Code	Food	Flora	s_{IS}	s_{tot}	$s_{rés}$	s_{cond}
1'	Sausage meat	aerobic mesophilic (2 dishes)	**0.14**	0.15	0.04	
1'	Sausage meat	aerobic mesophilic (1 dish)	**0.14**	0.15	0.06	
1'	Sausage meat	coliforms (2 dishes)	**0.01**	0.17	0.16	
1'	Sausage meat	coliforms (1 dish)	**0.01**	0.14	0.14	
1'	liver	aerobic mesophilic (2 dishes)	0.16	0.17	0.07	
1'	liver	aerobic mesophilic (1 dish)	0.17	0.18	0.07	
1'	liver	coliforms (2 dishes)	0.18	0.20	0.08	
1'	liver	coliforms (1 dish)	0.17	0.18	0.07	
2'	Sausage meat	aerobic mesophilic (2 dishes)	**0.02**	0.02	0.00	0.01
2'	Sausage meat	aerobic mesophilic (1 dish)	**0.02**	0.02	0.01	0.01
2'	Sausage meat	coliforms (2 dishes)	**0.01**	0.01	0.00	0.01
2'	Sausage meat	coliforms (1 dish)	**0.01**	0.01	0.01	0.01
2'	pork meat product	aerobic mesophilic (2 dishes)	0.73	0.73	0.02	0.01
2'	pork meat product	aerobic mesophilic (1 dish)	0.73	0.73	0.03	0.00
2'	pork meat product	coliforms (2 dishes)	0.60	0.60	0.04	0.04
2'	pork meat product	coliforms (1 dish)	0.59	0.60	0.06	0.04
3'	Sausage meat	aerobic mesophilic (2 dishes)	**0.05**	0.08	0.05	0.03
3'	Sausage meat	aerobic mesophilic (1 dish)	**0.06**	0.08	0.06	0.00
3'	Sausage meat	coliforms (2 dishes)	**0.09**	0.11	0.07	0.01
3'	Sausage meat	coliforms (1 dish)	**0.09**	0.12	0.08	0.00
3'	poultry leg	aerobic mesophilic (2 dishes)	0.40	0.41	0.07	0.07
3'	poultry leg	aerobic mesophilic (1 dish)	0.40	0.41	0.08	0.04
3'	poultry leg	coliforms (2 dishes)	0.51	0.52	0.10	0.02
3'	poultry leg	coliforms (1 dish)	0.51	0.52	0.11	0.00
4'	Sausage meat	aerobic mesophilic (2 dishes)	**0.12**	0.13	0.05	
4'	Sausage meat	aerobic mesophilic (1 dish)	**0.11**	0.13	0.07	
4'	Sausage meat	coliforms (2 dishes)	**0.00**	0.09	0.09	
4'	Sausage meat	coliforms (1 dish)	**0.05**	0.08	0.07	
5'	Sausage meat	aerobic mesophilic (2 dishes)	**0.08**	0.14	0.07	0.09
5'	Sausage meat	aerobic mesophilic (1 dish)	**0.08**	0.15	0.07	0.10
5'	Sausage meat	coliforms (2 dishes)	**0.16**	0.19	0.08	0.08
5'	Sausage meat	coliforms (1 dish)	**0.14**	0.19	0.10	0.08
5'	Shrimp raviolis	aerobic mesophilic (2 dishes)	0.11	0.14	0.05	0.06
5'	Shrimp raviolis	aerobic mesophilic (1 dish)	0.11	0.14	0.06	0.06
5'	Shrimp raviolis	coliforms (2 dishes)	0.14	0.18	0.07	0.08
5'	Shrimp raviolis	coliforms (1 dish)	0.13	0.17	0.08	0.09
6'	Sausage meat	aerobic mesophilic (2 dishes)	**0.06**	0.07	0.02	
6'	Sausage meat	aerobic mesophilic (1 dish)	**0.04**	0.05	0.04	
6'	Sausage meat	coliforms (2 dishes)	**0.04**	0.07	0.06	
6'	Sausage meat	coliforms (1 dish)	**0.06**	0.10	0.08	
6'	Pastries (Eclair)	aerobic mesophilic (2 dishes)	0.06	0.09	0.06	
6'	Pastries (Eclair)	aerobic mesophilic (1 dish)	0.07	0.09	0.07	
6'	Pastries (Eclair)	coliforms (2 dishes)	0.05	0.08	0.06	
6'	Pastries (Eclair)	coliforms (1 dish)	0.03	0.10	0.09	
7'	Sausage meat	aerobic mesophilic (2 dishes)	**0.06**	0.16	0.15	0.01
7'	Sausage meat	aerobic mesophilic (1 dish)	**0.07**	0.21	0.20	0.01
7'	Sausage meat	coliforms (2 dishes)	**0.19**	0.29	0.22	0.05
7'	Sausage meat	coliforms (1 dish)	**0.13**	0.28	0.25	0.01
8'	Sausage meat	aerobic mesophilic (2 dishes)	**0.03**	0.13	0.05	0.11
8'	Sausage meat	aerobic mesophilic (1 dish)	**0.04**	0.16	0.05	0.15
8'	Sausage meat	coliforms (2 dishes)	**0.13**	0.15	0.05	0.02
8'	Sausage meat	coliforms (1 dish)	**0.13**	0.15	0.06	0.00
8'	Raw milk	aerobic mesophilic (2 dishes)	0.01	0.10	0.04	0.09
8'	Raw milk	aerobic mesophilic (1 dish)	0.02	0.12	0.04	0.12
8'	Raw milk	coliforms (2 dishes)	0.03	0.09	0.06	0.05
8'	Raw milk	coliforms (1 dish)	0.03	0.09	0.06	0.06
9'	Sausage meat	aerobic mesophilic (2 dishes)	**0.07**	0.14	0.09	0.09
9'	Sausage meat	aerobic mesophilic (1 dish)	**0.07**	0.14	0.08	0.08
9'	Sausage meat	coliforms (2 dishes)	**0.20**	0.28	0.10	0.16
9'	Sausage meat	coliforms (1 dish)	**0.21**	0.28	0.10	0.16
9'	Tabbouleh	aerobic mesophilic (2 dishes)	0.20	0.23	0.10	0.05
9'	Tabbouleh	aerobic mesophilic (1 dish)	0.19	0.22	0.10	0.05
9'	Tabbouleh	coliforms (2 dishes)	0.02	0.08	0.05	0.06
9'	Tabbouleh	coliforms (1 dish)	0.03	0.08	0.05	0.06