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NF Validation certification of the 3M™ Petrifilm™ Staph Express System (STX system) for the "coagulase positive *Staphylococcus*" enumeration

Reference method EN ISO 6888-2

Certificate number: 3M – 01/09 – 04/03 B

Comparative and interlaboratory studies according to the EN ISO 16140 standard

SUMMARY REPORT

Validation date:	2003.04.02
Renewal date:	2007.03.29[1]
[1] *(EN ISO 16140 protocol and EN ISO 6888-2:1999)*	
	2011.02.03[2]
[2] *(EN ISO 16140/A1 protocol)*	
End validation date:	2015.04.02

Petrifilm STX vs RPFA – summary 2011 v01

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Contents

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1. Introduction

1.1 Certification references

The 3M™ Petrifilm™ Staph Express Count system (STX) has been validated in April 2003 with certificate number N°3M – 01/09 – 04/03 A for human food products, and according to EN ISO 6888-1:1999 with its 2004 amendment.
For the 2011 renewal study, the study was done in 2007 using EN ISO 6888-2:1999 (technique using rabbit plasma fibrinogen agar), and its 2003 amendment, as reference method and EN ISO 16140:2003 as reference protocol. This method certificate is 3M – 01/09 – 04/03 B.
For 2011 renewal, the 3M™ Petrifilm™ Staph Express Count system (STX) has been validated according to the EN ISO 16140/A1 (2010) with reinterpretation of collaborative study results.

1.2 Principle and protocol of the alternative method

1.2.1 Principle of the alternative method

The Petrifilm™ Staph Express Count System (STX system) consists of a Petrifilm™ Staph Express Count Plate (STX plate) and a Petrifilm™ Staph Express Disk (STX disk).

The Petrifilm™ Staph Express Count Plate is a sample-ready culture medium system which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for *Staphylococcus aureus, S. hyicus* and *S. intermedius*.

The Petrifilm™ Staph Express disk contains toluidine blue-O that facilitates the visualization of deoxyribonuclease (DNase) reactions. DNase-positive organisms detected on the Petrifilm™ Staph Express plate are *S. aureus*, *S. hyicus* and *S. intermedius*. These three organisms represent the majority of the group of organisms commonly known as coagulase-positive *staphylococci*.

1.2.2 Protocol

From a mother suspension realized according to the prescriptions of the EN ISO 6887 standard, or directly from a liquid sample, decimal dilutions are realized and 3M™ Petrifilm™ Staph Express Count system (STX) is inoculated as described below:

- Place the Petrifilm Staph Express Plate on a flat, level surface,
- Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension or dilution onto the center bottom film,
- Roll the top film down onto the sample to prevent trapping air bubbles,
- Place the plastic spreader with the flat side down on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire Petrifilm Plate growth area before the gel is formed.
- Remove the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.
- Incubate plates in a horizontal position with the clear side up in stacks of no more than 20 plates.

Petrifilm Plates are incubated for **24 hr ± 2 hr at 37°C ±1°C** .

After incubation, Petrifilm™ Staph Express Plates are counted with a standard colony counter or other illuminated magnifier according to colony colors observations.

- no colonies : the test is complete,
- observation of only red-violet colonies after 24 hr ± 2 hr : count these colonies as *S. aureus*, *S. hyicus* and *S. intermedius*, the test is complete.
- observation of any colony colors besides red-violet (for example : black or blue-green) : use a Petrifilm Staph Express Disk. Black colonies may be stressed microorganisms.

The Petrifilm™ Staph Express Count system (Petrifilm+Disk) is incubated for **3 hours at 37℃ ±1℃** .
Count all pink zones wether or not colonies are visible. Pink zones are usually associated with *S. aureus* but may indicate *S. hyicus* or *S. intermedius*. Colonies not associated with a pink zone are not DNase producing *Staphylococci*, and should not be counted. If the entire disked area is pink with no distinct zones, large numbers of DNase producing colonies are present. Record the result as too numerous to count (TNC) and dilute the sample further to obtain a more accurate count.

Calculate the number of microorganisms present in the test sample according to ISO 7218 for one plate per dilution.
Counting range is:

- less than or equal to 150 red-violet colonies and/or less than or equal to 300 total colonies
- less than or equal to 150 pink zones. (Note : read the plates after 3 hours of incubation time is complete).

Analytical diagrams are presented on figures 1 and 2.

Figure-1 : 3M™ Petrifilm™ Staph Express count plate

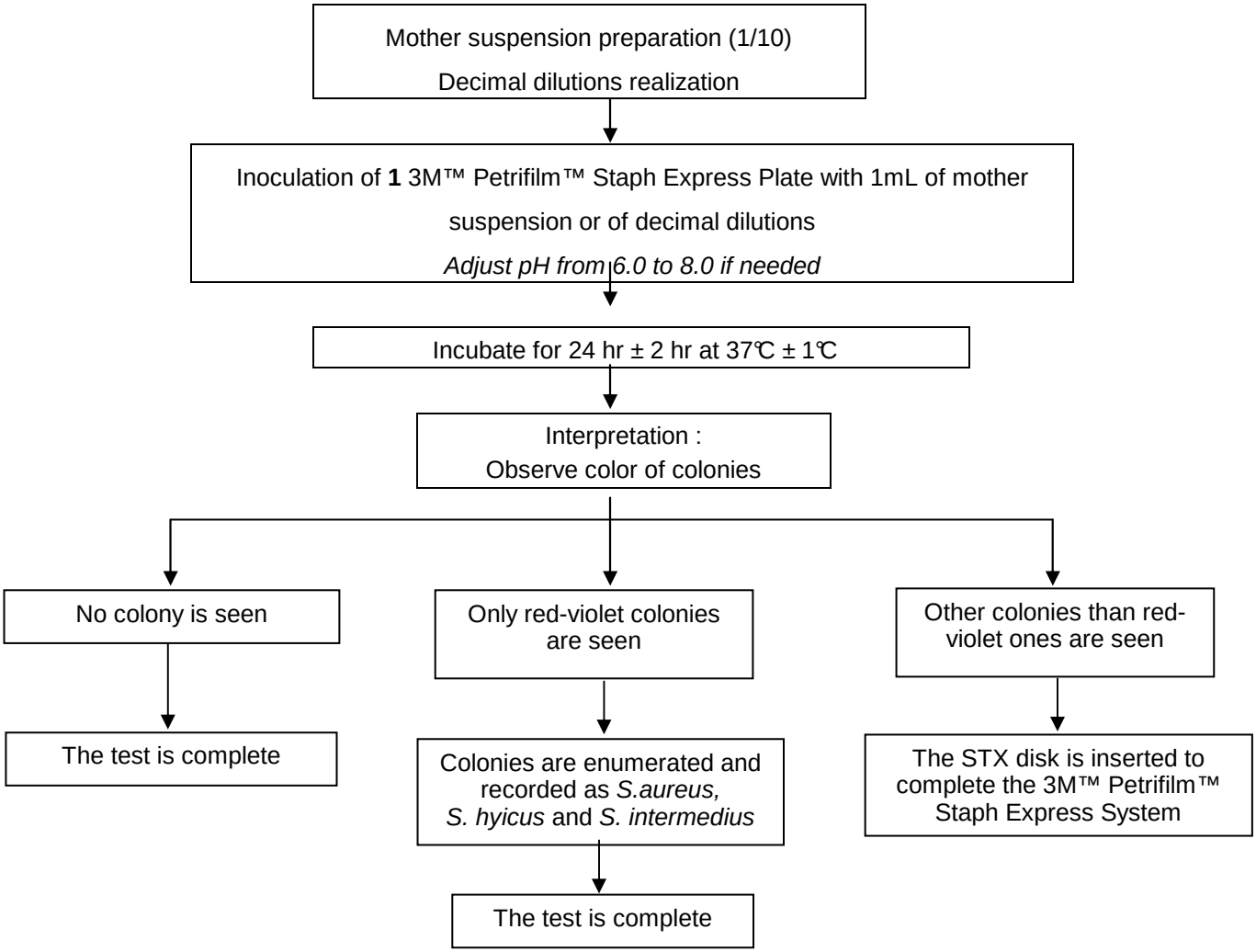
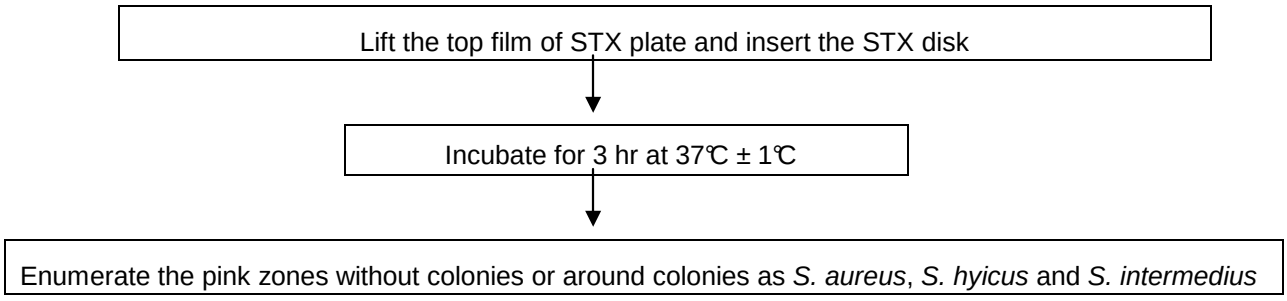


Figure 2 : 3M™ Petrifilm™ Staph Express Disk INSERTION



1.3 Application scope

The validation scope corresponds to all human food products and pet foods.

1.4 Reference method

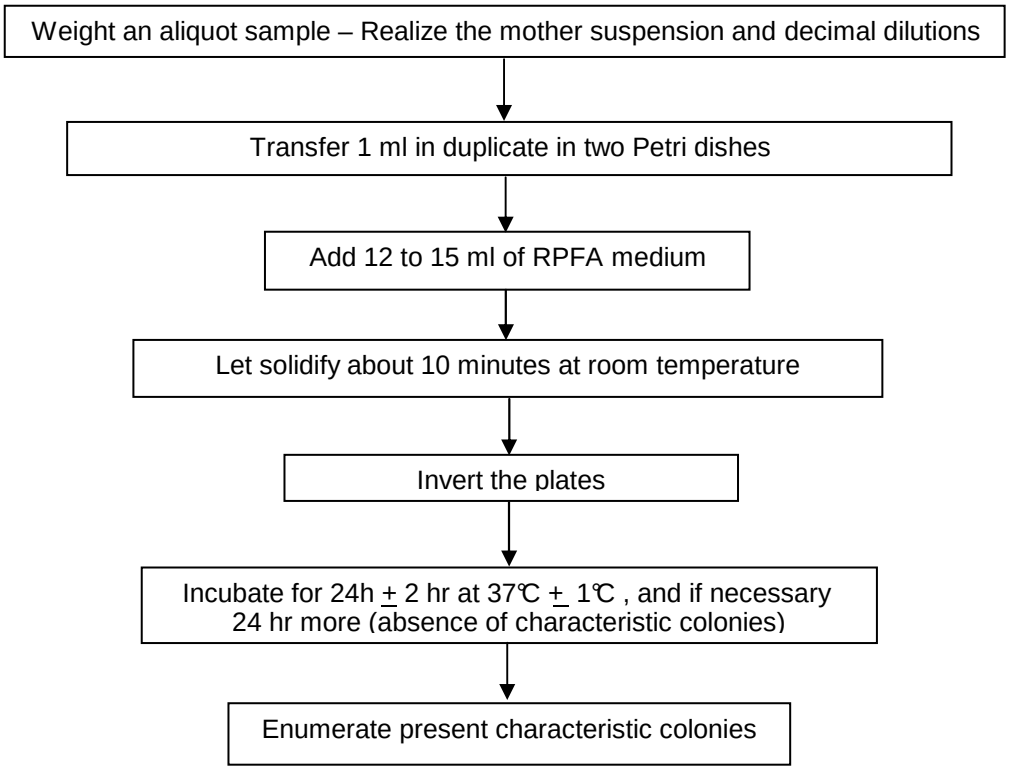
Initial validation (2007):

- EN ISO 6888-2:1999 - Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 2: Technique using rabbit plasma fibrinogen agar medium (or RPFA).
- EN ISO 6888-2/A1:2003 - Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive *staphylococci* (*Staphylococcus aureus* and other species) – Part 2: Technique using rabbit plasma fibrinogen agar medium. Amendment 1: Inclusion of precision data.

This method is a method without confirmation of the characteristic colonies using coagulase activity test.

Analytical diagram is presented on figure 3.

Figure 3: EN ISO 6888-2 Standard



1.5 Background of certification

The 3M™ Petrifilm™ Staph Express Count system (STX system) has been validated since April 2003 (certificate n°3M 01/09-04/03 **A**). The reference method was the EN ISO 6888-1:1999 standard and its 2004 amendment, including some accuracy data.

In 2007, the reference method was the EN ISO 6888-2:1999 standard (technique using rabbit plasma fibrinogen agar), and its 2003 amendment. This method certificate was 3M – 01/09 – 04/03 **B**. Some assays in comparison with the EN ISO 6888-2:1999 standard and with its amendment of 2003 were presented in 2008.

Results of the studies from 2003 were collected in the part « inclusivity/exclusivity ».

In 2007, the reference protocol was the EN ISO 16140 standard (2003). The alternative method has not been modified since 2007.

The 2011 renewal study takes into account the new amendment of the EN ISO 16140 standard, published in January 2010 [EN ISO 16140/A1: Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods – Amendment 1: Interlaboratory study on quantitative methods, including new calculations for Interlaboratory study].

The collaborative study was therefore reviewed to fulfil the new amendment (new statistical assessment).

2 Comparative study of methods

The following criteria were determined relative accuracy, linearity, inclusivity and exclusivity and practicability.

2.1 Relative accuracy

The relative accuracy is the closeness of agreement between a test result and the accepted reference value.

2.1.1 Number and nature of the samples

According to the EN ISO 16140 reference document, food products (naturally contaminated or spiked samples) have been analysed in duplicate according to the 2 methods :

- reference method EN ISO 6888-2, using RPFA medium,
- and STX system.

In total, 197 products were analyzed so as to obtain at least 10 usable results in each food category. Detailed results are presented in appendix A.
The categories and the types of samples studied are the following:

Categories	Types	Analyzed samples	Exploited results
Meat products	Raw meat	26	11
	Prepared & seasoned (raw) meat	12	7
	Charcuteries	17	6
	TOTAL	**55**	**24**
Milk products	Raw milk cheeses	30	8
	Raw milk and raw cream	14	7
	Ice cream	5	5
	TOTAL	**49**	**20**
Seafood products	Raw fish	10	5
	Shellfish	9	5
	Prepared fish	12	6
	TOTAL	**31**	**16**
Vegetables	Raw vegetables	4	2
	Seasoned vegetables	16	9
	Cooked vegetables	3	1
	TOTAL	**26**	**12**
Pastries	With butter cream	8	6
Egg products	With custard	6	3
	Egg products	3	1
	TOTAL	**17**	**10**
Petfood	Dry food	14	7
	Raw meat	4	4
	Cat/dog food	4	2
	TOTAL	**22**	**13**
TOTAL		**197**	**95**

The 102 samples the results of which were not used, exhibited :

- Colony counts below 10 CFU/g or 100 CFU/g with both methods in 58 cases,
- Colony counts below 10 CFU/g or 100 CFU/g with one method in 21 cases,
- Non interpretable results in 23 cases.

2.1.2 Artificial contamination of the samples and percentage

Artificial contamination was achieved on 14 samples by using stressed contaminating suspensions, the stress treatment and efficiency of which have been determined.
The percentage of artificial contamination was globally **15%** for the samples with interpretable results.

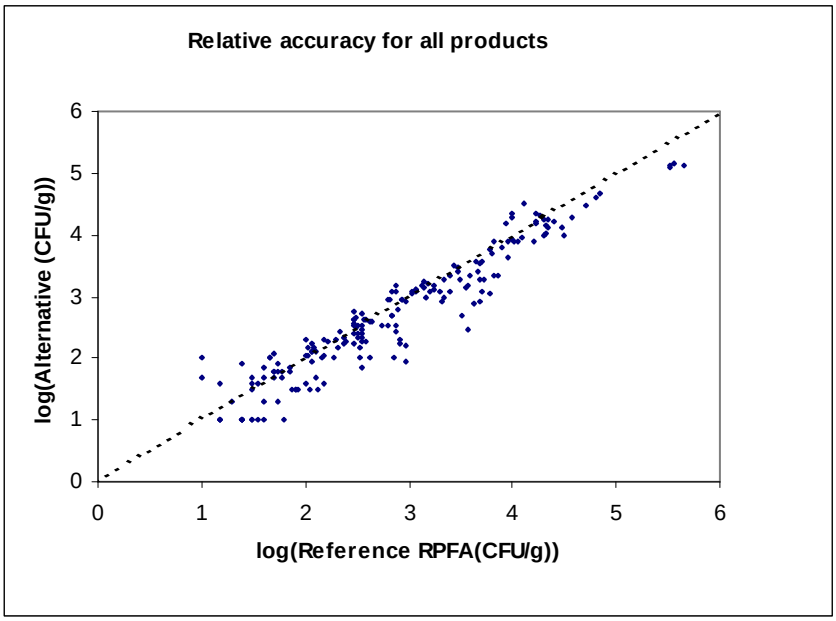
2.1.3 Raw data

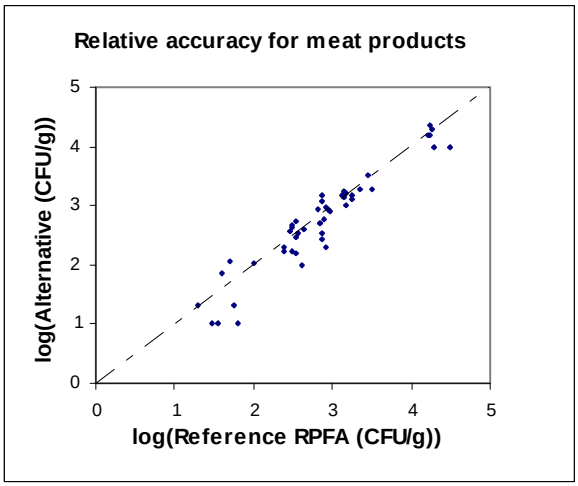
Each sample was analyzed in duplicate by the alternative method and the reference method.

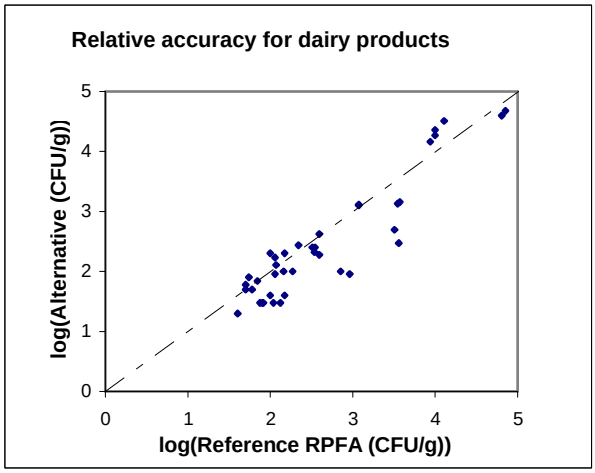
Following the EN ISO 16140 standard, the values for each sample were plotted on a two-dimensional graph. The vertical axis (y) is used for the alternative method and the horizontal axis (x) for the reference method.

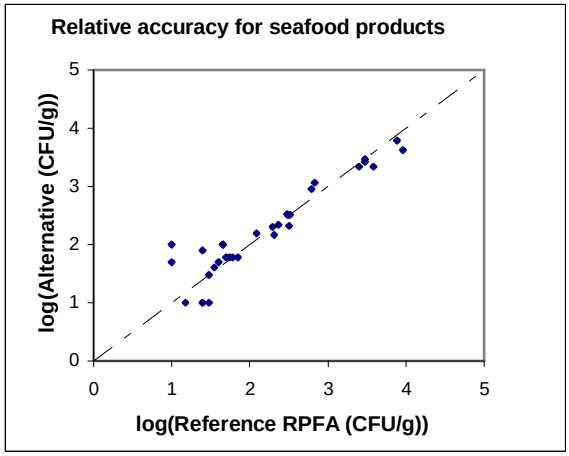
The data were then tested by a linear regression program in order to determine the intercept value (a) and the slope value (b).
The relative accuracy relationship is evaluated according to the model: y = bx + a.
For each of the two methods, robust repeatability standard deviations were calculated (Rob.sr(x) and Rob.sr(y)).
As a function of the ratio of these standard deviations, Rob.R = Rob.sr(y)/Rob.sr(x), the linear regression to be used for the interpretation is defined in the EN ISO 16140 standard.

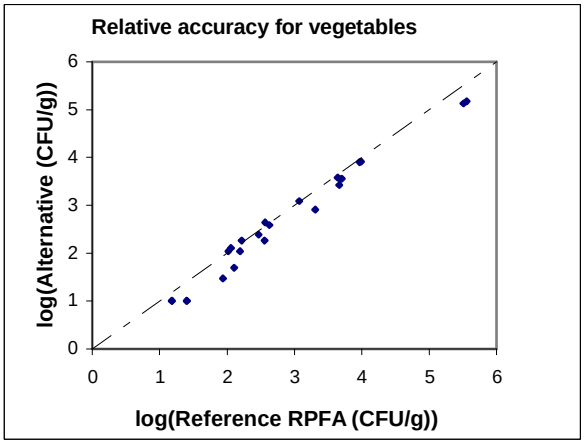
The following graphs represent the raw values obtained for the samples analyzed for all products and in each category.
The straight line represented is the first bisector (y = x).

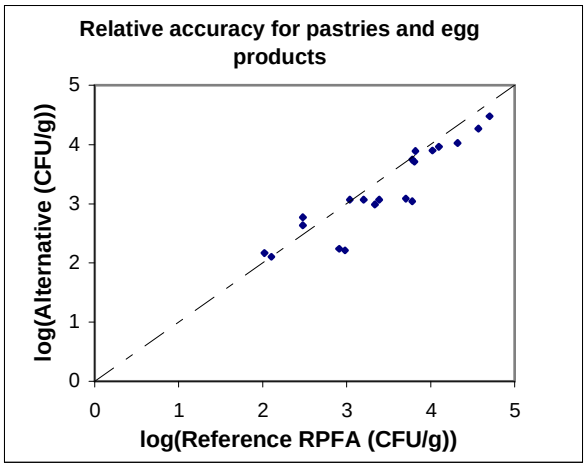


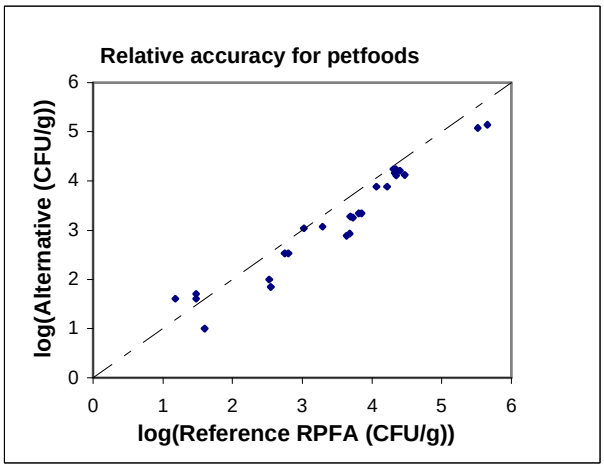












2.1.4 Statistical interpretation

In order to check whether the relative accuracy is satisfactory, the two following hypotheses must be verified for a risk α = 5%:

- **Ordinate at the origin (or intercept) {a = 0}**
  The alternative method exhibits a systematic bias compared with the reference method:
  - If the value t = a / Sa with (q-2) degrees of freedom is higher than the T-critical value, obtained in Student's table, or
  - If the probability p{a = 0} < α (=0.05), p{a = 0} being defined by Student's law.
- **Slope {b = 1}**
  If the alternative method does not yield the same values as the reference method:
  - The value t = (b-1) / Sb with (q-2) degrees of freedom is higher than the T-critical value, obtained in Student's table, or
  - If the probability p{b = 1} < α (=0,05), p{b = 1} being defined by Student's law.

Different values needed in the EN ISO 16140 standard are clarified in table below. It allowed to compare 3M™ Petrifilm™ Staph Express enumeration system with reference method.

Matrix	Rob.R	Regression used	a	t(a)	p(t ;a=0)	b	t(b)	p(t ;b=1)	Conclusion
Meat products	0.848	GMFR	-0.333	2.026	0.055	1.081	1.451	0.161	{a=0} accepted {b=1} accepted
Milk products	1.171	GMFR	-0.432	1.652	0.116	1.090	0.962	0.348	{a=0} accepted {b=1} accepted
Seafood products	1.368	GMFR	+0.176	0.977	0.345	0.851	1.283	0.409	{a=0} accepted {b=1} accepted
Vegetables	0.818	GMFR	-0.178	1.488	0.168	1.000	0.008	0.994	{a=0} accepted {b=1} accepted
Pastries- Egg products	0.784	GMFR	-0.047	0.098	0.924	0.954	0.341	0.742	{a=0} accepted {b=1} accepted
Petfood	0.696	GMFR	-0.089	0.382	0.710	0.934	1.055	0.314	{a=0} accepted {b=1} accepted
All products	**1.044**	**GMFR**	**-0.083**	**0.981**	**0.329**	**0.979**	**0.760**	**0.449**	**{a=0} accepted {b=1} accepted**

The equation for the regression lines between the alternative method and the reference method, for each category of products are the following table:

Matrix	Equation
Meat products	log (Alt) = 1.0813 log (Ref) – 0.3331
Milk products	log (Alt) = 1.0900 log (Ref) – 0.4317
Seafood products	log (Alt) = 0.9347 log (Ref) + 0.1760
Vegetables	log (Alt) = 1.0003 log (Ref) – 0.1779
Pastries - Egg products	log (Alt) = 0.9539 log (Ref) - 0,0466
Petfood	log (Alt) = 0.9338 log (Ref) – 0,0886
All products	**log (Alt)= 0.9787 log(Ref) – 0.0827**

The graph representing the regression lines obtained (y = bx + a), with the vertical axis (y) used for the alternative method and the horizontal axis (x) for the reference method, for all products, is presented in appendix B.

Other parameters were presented in the following tables:

- the limits of robust repeatability (log values) obtained for the alternative method and the reference method
- the bias between the two methods (alternative method –reference method)

Matrix	Robust repeatability		Bias (D) (log CFU/g) (alternative – reference)		Contamination range (log)
	Ref.	Alt.	average	median	
Meat products	0.25	0.21	-0.102	-0.086	1.00 - 4.36
Dairy products	0.28	0.32	-0.193	-0.127	1.48 - 4.85
Seafood products	0.26	0.35	+0.033	-0.046	1.00 - 3.96
Vegetables	0.17	0.14	-0.186	-0.187	1.00 - 5.56
Pastries – Egg products	0.22	0.17	-0.204	-0.121	2.10 - 4.70
Pet foods	0.15	0.10	-0.321	-0.293	1.00 - 5.63
All products	**0.22**	**0.23**	**-0.143**	**-0.126**	**1.00 - 5.63**

2.1.5 Conclusion

For all product categories, the two hypotheses {a=0} and {b=1} are accepted. There is no systematic bias between the two methods.
The repeatability log values obtained with the alternative method and the reference method are 0.23 for the alternative method and 0.22 for the reference method.
The bias calculated between the alternative method and the reference method is in the order of D= – 0.13 log (alternative method –reference method).

2.2 Linearity

Linearity is the ability of the method when used with a given matrix to give results that are in proportion to the amount of analyte present in the sample, that is an increase in analyte corresponds to a linear or proportional increase in results.

2.2.1 Nature of the tests

Five food products were contaminated, at five contamination levels. For each product and each contamination level, the alternative and the reference methods were performed with two repetitions.

The analysed products were the following:

- raw grounded meat,
- raw milk,
- raw fish,
- shredded carrots,
- pet food.

The contamination level were:

100 to 500	CFU/g
500 to 1000	CFU/g
1000 to 5000	CFU/g
5000 to 10 000	CFU/g
10 000 to 100 000	CFU/g

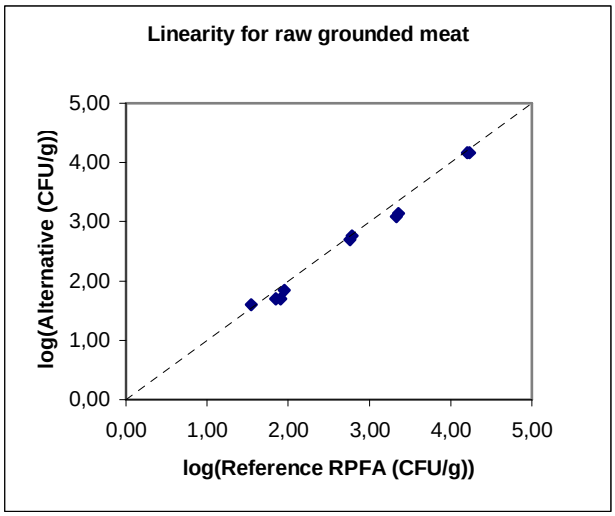
Different strains of *Staphylococcus aureus* were used, as presented in the following table:

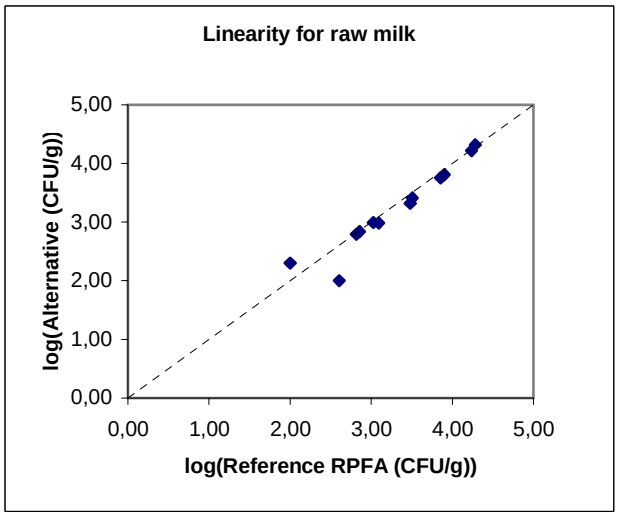
Product	Strain and origin
Raw grounded meat	*Staphylococcus aureus* from grounded raw meat
Raw milk	*Staphylococcus aureus* from yoghurt
Raw fish	*Staphylococcus aureus* from smoked salmon
Shredded carrots	*Staphylococcus aureus* from vegetables salad
Pet food	*Staphylococcus aureus* from meat product

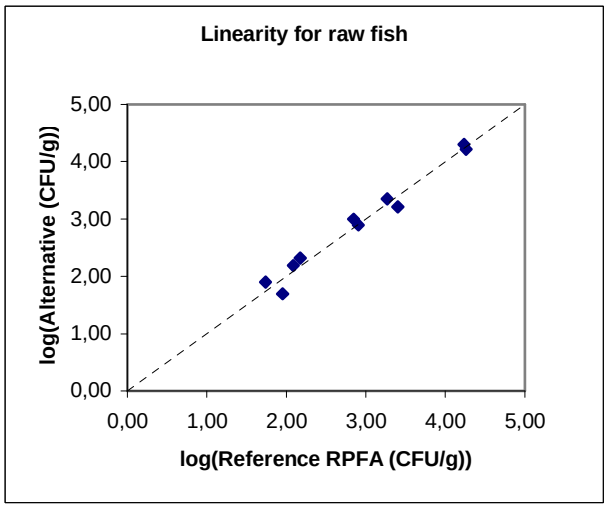
2.2.2 Raw data

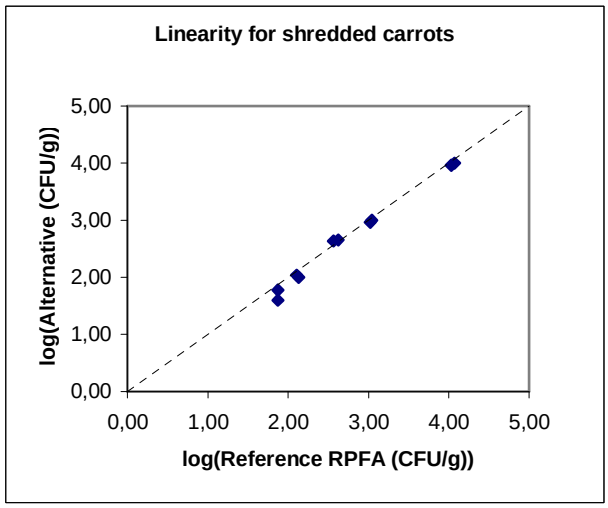
Following the EN ISO 16140 standard, the values for each sample were plotted on a two-dimensional graph. The vertical axis (y) is used for the alternative method and the horizontal axis (x) for the reference method. The data were then tested by a linear regression program in order to determine the intercept value (a) and the slope value (b), like in the relative accuracy part.

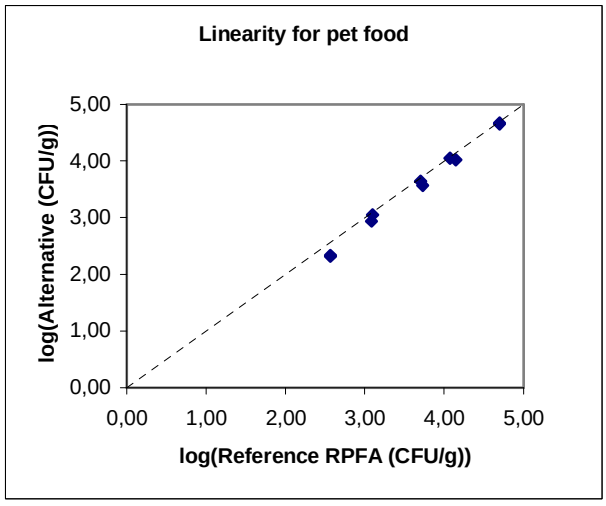
The following graphs represent the raw values obtained for each product.











2.2.3 Statistical Interpretation

The linearity is evaluated with the probability of lack-of-fit.
The value Rob.F is calculated as follow:

$$Rob.F = \frac{(N-2)\,(s^2y{:}x \,/\, Rob.sr(y)^2) - q\,(n-1)}{q-2}$$

with q, number of levels (q = 5)
n, number of repetitions (n = 2)
N, number of samples (N = nq)

The relationship is non linear if:

- [Rob.F > Fcrit (νnum, νden)]

or

- p(F, νnum, νden).< α (=0.05)

The type of regression and the Rob.F values are detailed in the following table:

Product	**Rob.R**	**Régression used**	**F critical value**	**Rob.F**	**p (Rob.F) %**
Raw grounded meat	2.195	OLS	5.41	10.122	1 %
Raw milk	1.643	GMFR	5.19	1.751	25 %
Raw fish	1.506	GMFR	5.41	0.363	78 %
Shredded carrots	1.094	GMFR	5.41	38.225	0 %
Pet food	1.607	GMFR	5.41	11.321	1 %

The equations for the regression lines between the alternative method and the reference method, are the following:

Raw grounded meat	log Alt = 0.9958 log Ref - 0.0945	R^2 = 0.991
Raw milk	log Alt = 1.0306 log Ref - 0.1781	R^2 = 0.995
Raw fish	log Alt = 0.9885 log Ref + 0.0529	R^2 = 0.994
Shredded carrots	log Alt = 1.0418 log Ref - 0.1828	R^2 = 0.992
Pet food	log Alt = 1.0789 log Ref- 0.3991	R^2 = 0.998

2.2.4 Conclusion

The statistical tests conclude that the relationship between the alternative method and the reference method is linear for “raw milk” and “raw fish”.
For « raw grounded meat », « shredded carrots », « pet food », the non-linearity test is highly significant.
But, the correlation coefficients for these three products are very high, about 99%, so the significance of the non-linearity test could be failed.

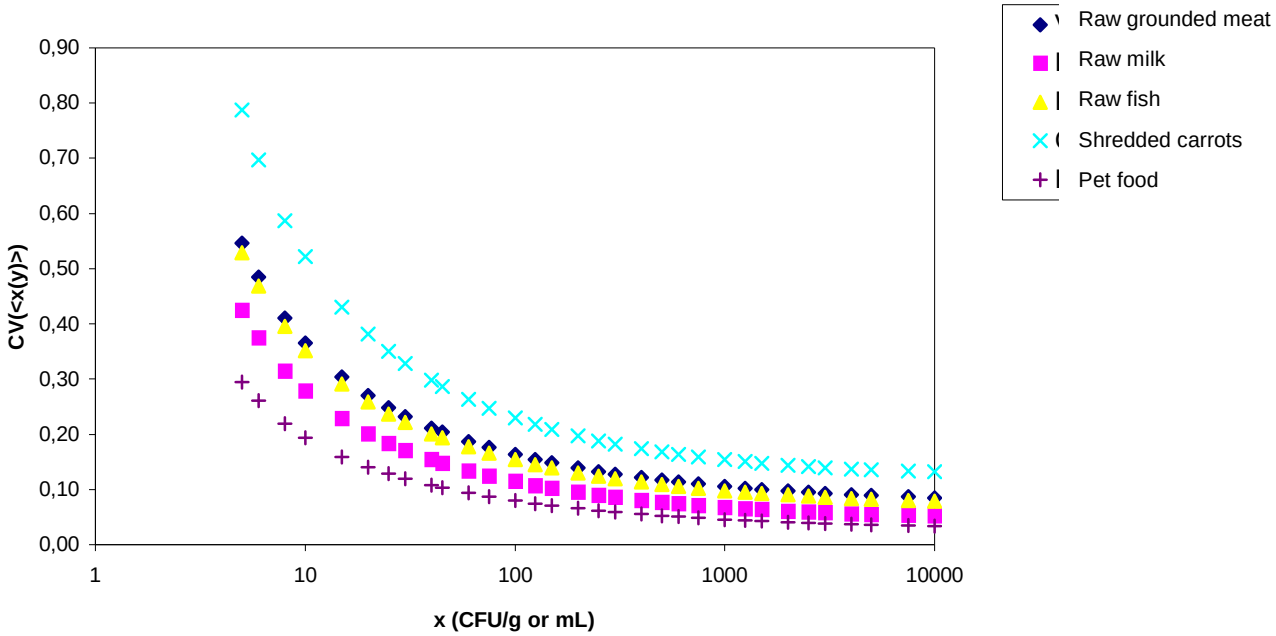
And considering the different graphs and regression equations, the **linearity is satisfactory**.

2.3 Relative sensitivity and determination of unknown samples

An estimate of sensitivity is used in EN ISO 16140 in order to ascertain that values given by the alternative method do not differ markedly from the reference method (less than 30% in difference).
The relative sensitivity is defined as the ability of the alternative method within a given matrix, at a specified average value, or over the whole measurement range. It is the minimal quantity variation (increase of the analyte concentration x) which gives a significant variation of the measured signal (response y).

For each matrix, the precision profile s(<x(y)>) or CV(<x(y)>) versus x(y) is determined over the whole measurement range.

Precision profiles



2.4 Detection and quantification limits

Detection (LOD) and quantification (LOQ) limits were determined by analysis of pure culture *Staphylococcus aureus* with alternative method.

Four levels of inoculation were tested, with six replicates by level.
Results are synthesized in tables below.

Level / ml	Positive samples number	Standard deviation s_0	Bias x_0 (x_{0i} median)
0	0/6		
0.93	2/6	0.00	0.0
1.24	3/6	0.00	0.5
1.86	4/6	1.19	1.5
12.4	6/6	13.12	11.5

From s_0 and x_0 values obtained for the first level, the critical limit (LC), the detection limit (LOD) and the quantification limit (LOQ) were determined:

	Formula	Value obtained (CFU/ml)
LC	1.65 $\mathbf{s_0}$ + $\mathbf{x_0}$	3.7
LOD	3.3 $\mathbf{s_0}$ + $\mathbf{x_0}$	5.4
LOQ	10 $\mathbf{s_0}$ + $\mathbf{x_0}$	13.4

2.5 Specificity / selectivity

The aim of this study is to check that all coagulase positive *Staphylococcus* strains are detected, and that no cross reaction exists with other species of *Staphylococcus* (except *S. hyicus* and *S. intermedius*) or with other genus strains.

2.5.1 Protocol

Strains have been cultivated in brain heart infusion during 18 to 24 hours at 37°C.
Different dilutions are realized and inoculated on 3M™ Petrifilm™ Staph Express test and on Baird Parker agar medium.
To study the system specificity, whatever the utilization conditions, the disk was inserted in all cases. The growth, the coloring and the DNase reaction of colonies were observed.

2.5.2 Results and conclusion

Results are listed in appendix C.

✓ All 28 *Staphylococcus aureus* tested strains gave red-violet colonies.
After disk insertion, all colonies were surrounded with a pink zone.

✓ The other *Staphylococci*, which are coagulase-positive strains, *S. hyicus* and *S. intermedius*, presented typical aspect as *Staphylococcus aureus:* the colonies were red-violet or dark, and after revelation with STX disk, the colonies were surrounded with a pink zone.

✓ Other 15 *Staphylococci* tested strains, which are not coagulase-positive strains and the 11 strains of other genus didn't give any culture or typical colonies (no red-violet color, no pink zone after disk insertion).

Inclusivity and exclusitivity are **satisfactory**.
3M™ Petrifilm™ Staph Express enumeration system permitted to detect all coagulase-positive *Staphylococcus* inoculated strains. All colonies had a typical aspect after incubation and after revelation with STX disk.

3 Interlaboratory study

The aim of the interlaboratory study was to determine the variability of the results obtained in different laboratories using identical samples and to compare these results with those obtained during the methods comparison study.

The interlaboratory study was conducted in 2007 according to EN ISO 16140 (2003).
A new statistical assessment was done in 2011 (renewal study) based on all data collected in 2007 **according to the EN ISO 16140 (amendment 1, 2010) standard**.

3.1 Study organization

Thirteen (13) laboratories took part in the interlaboratory study.
Pasteurized milk has been inoculated by a coagulase-positive *Staphylococcus aureus* strain, isolated from dairy product.
Height samples were prepared per laboratory, two flasks par inoculation level.
Interlaboratory study laboratories and the expert laboratory have carried out the analyses with the alternative and reference methods.
The analyses have been performed two days after sending the samples.
According to temperature conditions, the results of 12 laboratories have been included to the statistical interpretations.

3.2 Control of experimental parameters

3.2.1 Contamination levels obtained after artificial inoculation

The four contamination levels are presented in the following table:

Level	Sample	Targeted level (CFU/ml)	Real level (CFU/ml))
Level 0	1 and 8	0	0
Level 1	2 and 7	100	81
Level 2	3 and 6	1 000	810
Level 3	4 and 5	10 000	8100

3.2.2 Strain stability during transport

In order to evaluate the *Staphylococcus aureus* strain variability during transport, bacterial count of inoculated milk at level 2, has been checked at different time, during storage at 7°C.
Results (CFU/ml) are reported in following table:

	J0	J1	J2
Sample 1	1400	1500	2000
Sample 2	1500	1400	1900
Sample 3	1900	2000	1700

No evolution of the strain has been observed after 48 h of storage at 7°C.

3.2.3 Problems of temperature recorded during transport, temperature on reception an reception times

The temperatures during transport have been registered and checked in order to verify their stability.
All temperature probes showed a temperature between 0°C and 8°C.
Measured temperatures on receipt are listed in following table:

Laboratory	Receipt Temperatures (°C)		Comments
	Measured by the laboratory	Temperature probe record	
A	6.5	1.9	/
B	5.0	2.4	/
C	1.2	2.4	/
D	3.0	0.4	/
E	1.7	0.0	/
F	4.0	4.5	/
G	9.4	7.9	Receipt at D+2
H	5.0	4.9	/
I	**12.3**	4.4	/
J	/	3.0	/
K	3.7	3.5	/
L	1.5	1.9	/
M	0.7	1.4	/

3.2.4 Conclusion

The laboratory G received the samples at D+2, the day when the labs had to carry out the analyses. The temperature checking showed that, for his package, the temperature stayed below 8°C.
The laboratory I announced a temperature higher as 8°C, but the temperature probe showed a temperature of 4,4°C on receipt.
The conditions of temperature for these two labs were within the correct range, so their results have been exploited.

The temperature curve during the storage after receipt and before performing the analyses was above the correct range: temperature upper than 8°C. So the results o f laboratory M had not been exploited.

Finally, according to temperature conditions, the results of 12 laboratories have been included to the statistical interpretations.

3.3 Results

3.3.1 Expert laboratory

Results obtained by the expert laboratory with EN ISO 6888-2 method (EN ISO 6888-2:1999 and EN ISO 6888-2/A1:2003) and Petrifilm™ Staph Express system are presented in the following table :

	EN ISO 6888-2		STX system	
	Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
Level 0	<10	<10	<10	<10
Level 1	95	30	40	40
Level 2	650	620	370	600
Level 3	6700	6700	6100	6200

Results according to standard EN ISO 6888-2 and alternative method were in agreement.

3.3.2 Results obtained by collaborative laboratories

Results of the 12 laboratories which realized the analysis are presented in the tables below:

Level 0 (results in CFU/ml)

Lab	EN ISO 6888-2		STX system	
	Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
A	<10	<10	<10	<10
B	<10	<10	<10	<10
C	<10	<10	<10	<10
D	<10	<10	<10	<10
E	<10	<10	<10	<10
F	<10	<10	<10	<10
G	<10	<10	<10	<10
H	<10	<10	<10	<10
I	<10	<10	<10	<10
J	<10	<10	<10	<10
K	<10	<10	<10	<10
L	<10	<10	<10	<10
M	Not interpretable	Not interpretable	Not interpretable	Not interpretable

Level 1 (results in CFU/ml)

Lab	EN ISO 6888-2		STX system	
	Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
A	70	55	80	100
B	110	65	40	90
C	85	90	40	90
D	75	75	70	90
E	80	70	40	40
F	55	60	70	60
G	85	65	30	40
H	100	Not interpretable	80	60
I	110	95	80	170
J	100	91	110	70
K	110	100	50	50
L	90	118	90	60
M	Not interpretable	Not interpretable	<10	60

Level 2 (results in CFU/ml)

Lab	EN ISO 6888-2		STX system	
	Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
A	780	870	590	720
B	580	680	500	620
C	1000	1000	580	710
D	1000	1000	780	830
E	840	810	560	680
F	990	860	670	710
G	930	720	630	670
H	Not interpretable	880	920	860
I	900	970	600	860
J	720	900	520	540
K	890	1100	680	620
L	950	1000	680	740
M	Not interpretable	Not interpretable	740	600

Level 3 (results in CFU/ml)

Lab	EN ISO 6888-2		STX system	
	Duplicate 1	**Duplicate 2**	**Duplicate 1**	**Duplicate 2**
A	8800	10000	7900	4600
B	8100	7200	5500	4800
C	16000	14000	9700	7600
D	11000	11000	8500	10000
E	7900	6900	5500	5800
F	8500	9600	4100	6300
G	7900	8600	6500	7500
H	Not interpretable	30000	8500	8900
I	9200	11000	11000	10000
J	9000	11000	5200	5100
K	8500	8000	6300	7100
L	10000	10000	8400	7500
M	Not interpretable	Not interpretable	8300	8600

3.3.3 Conclusion

For the laboratory M, the results are presented but have not been taken into account because of temperature problems during storage of the samples. In addition, this lab encountered problems using the standard method. But their results obtained with the alternative method were correct.

The laboratory H encountered problem for the interpretation of the dishes due to the interfering flora with the standard method. No result has been taken into account for this lab.

Therefore, **results of 11 laboratories** have been **statistically exploited**.

3.4 Calculations

Statistical interpretations have been calculated according to EN ISO 16140: 2003 and amendment 1, 2010 (EN ISO 16140/A1: 2010 document), per level of contamination.
Results were converted in log for the calculations.

3.4.1 Determination of the accuracy and fidelity characteristics

The application of the EN ISO 16140:2003 standard and of its amendment entails to determine the “robust values” to avoid excluding laboratories presenting extreme values.
The synthesis of obtained values is presented in the tables below.

			Reference method	Alternative method
Level 1	Unbiased Rousseeuw’s scale estimator Q_{intra}	$Q_{intra} = C_{2p}.Q_{2p}$	0.036	0.094
	Unbiased Rousseeuw’s scale estimator Q_{inter}	$Q_{inter} = C_{p}.Q_{p}$	0.090	0.168
	Mediane **m** :	**m**	1.920	1.820
	Standard deviation of repeatability S_r :	$S_r = \sqrt{2}\ Q_{intra}$	0.050	0.134
	Relative standard deviation of repeatability RSD_r :	$RSD_r = S_r / m$	0.026	0.074
	Repeatability limit ***r***:	$r = 2.8\ S_r$	0.141	0.375
	Interlaboratory standard deviation S_L:	$S_L = \sqrt{(Q^2_{inter} - Q^2_{intra})}$ *or* $S_L = 0$ if $Q^2_{inter} - Q^2_{intra} < 0$	0.083	0.138
	Standard deviation of reproducibility S_R :	$S_R = \sqrt{(S_L^2 + S_r^2)}$	0.097	0.192
	Relative standard deviation of reproducibility RSD_R :	$RSD_R = S_r / m$	0.051	0.106
	Reproducibility limit ***R***:	$R = 2.8\ S_R$	0.272	0.539
Level 2	Unbiased Rousseeuw’s scale estimator Q_{intra}	$Q_{intra} = C_{2p}.Q_{2p}$	0.026	0.038
	Unbiased Rousseeuw’s scale estimator Q_{inter}	$Q_{inter} = C_{p}.Q_{p}$	0.041	0.069
	Mediane **m** :	**m**	2.970	2.820
	Standard deviation of repeatability S_r :	$S_r = \sqrt{2}\ Q_{intra}$	0.036	0.054
	Relative standard deviation of repeatability RSD_r :	$RSD_r = S_r / m$	0.012	0.019
	Repeatability limit ***r***:	$r = 2.8\ S_r$	0.102	0.150
	Interlaboratory standard deviation S_L:	$S_L = \sqrt{(Q^2_{inter} - Q^2_{intra})}$ *or* $S_L = 0$ if $Q^2_{inter} - Q^2_{intra} < 0$	0.032	0.058
	Standard deviation of reproducibility S_R :	$S_R = \sqrt{(S_L^2 + S_r^2)}$	0.049	0.079
	Relative standard deviation of reproducibility RSD_R :	$RSD_R = S_r / m$	0.016	0.028
	Reproducibility limit ***R***:	$R = 2.8\ S_R$	0.136	0.220
Level 3	Unbiased Rousseeuw’s scale estimator Q_{intra}	$Q_{intra} = C_{2p}.Q_{2p}$	0.017	0.038
	Unbiased Rousseeuw’s scale estimator Q_{inter}	$Q_{inter} = C_{p}.Q_{p}$	0.080	0.138
	Mediane **m** :	**m**	3.980	3.850
	Standard deviation of repeatability S_r :	$S_r = \sqrt{2}\ Q_{intra}$	0.024	0.054
	Relative standard deviation of repeatability RSD_r :	$RSD_r = S_r / m$	0.006	0.014
	Repeatability limit ***r***:	$r = 2.8\ S_r$	0.066	0.150
	Interlaboratory standard deviation S_L:	$S_L = \sqrt{(Q^2_{inter} - Q^2_{intra})}$ *or* $S_L = 0$ if $Q^2_{inter} - Q^2_{intra} < 0$	0.079	0.133
	Standard deviation of reproducibility S_R :	$S_R = \sqrt{(S_L^2 + S_r^2)}$	0.082	0.143
	Relative standard deviation of reproducibility RSD_R :	$RSD_R = S_r / m$	0.021	0.037
	Reproducibility limit ***R***:	$R = 2.8\ S_R$	0.230	0.400

3.4.2 Control of the coherence of the results of measurement

Two graphic techniques of coherence are applied to identify the results of measurement or the inconsistent laboratories with regard to the other results of measurement or the laboratories: the statistics of coherence interlaboratory *h* and intra-laboratory *k* of Mandel.

In case of interlaboratory coherence, only 5 % or 1 % respectively *h* values are over the horizontal lines representing the indicators of this statistics.
In case of intra-laboratory coherence, only 5 % or 1 % respectively *k* values are over the horizontal lines representing the indicators of this statistics.

Figures showing the *h* and *k* plots for the for the reference method and the alternative method are presented in appendix D. Horizontal lines at the 5% and 1% significance indicators are added.

For the reference method, the robust analysis indicates some inconsistencies between measurement results or laboratories for interlaboratory coherence (*h* values):
- at level 1, no laboratory over thresholds,
- at level 2, 1 laboratory is over 5% and 1% threshold,
- at level 3, 1 laboratory is over 5% threshold.

For the alternative method, the robust analysis does not indicate inconsistencies between measurement results or laboratories for interlaboratory coherence (*h* values).

For the reference method and the alternative method, the robust analysis indicates some inconsistencies between measurement results or laboratories for intralaboratory coherence (*k* values).
. Reference method:
- at level 1, 1 laboratory is over 5% t and 1% thresholds,
- at level 2, 2 laboratories are over 5% threshold,
- at level 3, 3 laboratories are over 5% threshold.

. Alternative method:
- at level 1, 2 laboratories are over 5% threshold,
- at level 2, 1 laboratory is over 5% threshold,
- at level 3, 2 laboratories are over 5% threshold, including 1 laboratory over 1% threshold,.

The alternative method showed **satisfactory interlaboratory coherence**, slightly better than the reference method. **Intralaboratory results** are **comparable and less satisfactory for both methods**, although no laboratory showed systematically bad intralaboratory performance for all levels.

3.4.3 Bias calculation

For each level, difference between duplicate means obtained by the alternative method and the reference method has been calculated, that allows the determination of **bias D_{ij}** ($D_{ij} = y^*_{ij,\ Alt} - y^*_{ij,\ Ref.}$), with **y*** = mean of two values of measurement.

In order to verify if the relative accuracy is correct, the following {D=0} hypothesis was tested for each level, with calculating the statistic as:

$$t(d) = \frac{|\text{mediane}_i\ (Dij)|}{\sqrt{\pi/(2p)}\,Q_{diff}}$$

The bias is significant if the t(d) > 2, i;e; the alternative method lacks accuracy, relative to the reference method for the considered level.

The bias D (alternative-reference) values and the t(d) values obtained by level are reported in the following table:

	Biais (Dij) (log)	**t(d)**	**Conclusion Biais (alternative – reference)**
Level 1	-0.15	2.99	{D=0} refused
Level 2	-0.10	2.21	{D=0} refused
Level 3	-0.12	2.41	{D=0} refused

Reminder :
The bias value (alternative – reference) obtained during the comparative study was (– 0.13 log CFU/g).

Conclusion :
For the three higher levels, the {D=0} hypothesis is statistically refused. But, the bias values are similar to those obtained in the comparative study. So, the alternative method accuracy, relative to the reference method is satisfactory.

3.4.4 Repeatability calculation

For each method and each level, the repeatability limit r has been computed: r= 2.8 S_r, with S_r: repeatability standard deviation, Sr_{alt} for alternative method and Sr_{ref} for reference method.

So, for each level, repeatability standard deviations (Sr_{alt} and Sr_{ref}) were calculated.
If at level *j*, the standard deviation report ($S_{rj,\ Alt}/S_{rj,\ Ref}$) is upper to 2, fidelity of alternative method in repeatability conditions is considered as lower than that of the reference method.
If this report is lower than 0.5, fidelity of alternative method in repeatability conditions is considered as upper as that of the reference method.

Values obtained for the repeatability limit are reported in the following table:

	Repeatability limits r (log CFU/ml)		**Sr_{alt}/ Sr_{ref}**	**Conclusion**
	Reference method	**Alternative method**		
Level 1	0.141	0.375	2.65	Different repeatability
Level 2	0.102	0.150	1.47	Equivalent repeatability
Level 3	0.067	0.150	2.27	Different repeatability

Standard deviation reports of repeatability are included between 1.47 and 2.64; at all three levels, they are larger than 0.5 and smaller than 2.
The alternative method and the reference method repeatability limits are statistically comparable for medium level (L2) and different (lower) for low (L1) and high (L3) levels.

Reminder :
The repeatability limits r values obtained during the comparative study were about 0.22 log (CFU/g) for reference method and 0.23 (CFU/g) for alternative method.

3.4.5 Reproducibility calculation

For each method and each level, the reproducibility limit r has been computed: R= 2.8 S_R, with S_R: reproducibility standard deviation, S_{Ralt} for alternative method and S_{Rref} for reference method.

So, for each level, reproducibility standard deviations (Sr_{alt} and Sr_{ref}) were calculated.
If at level *j*, the standard deviation report ($S_{R,\ Alt}/S_{Rj,\ Ref}$) is upper to 2, fidelity of alternative method in reproducibility conditions is considered as lower than that of the reference method.
If this report is lower than 0.5, fidelity of alternative method in reproducibility conditions is considered as upper than that of the reference method.

Values obtained for the reproducibility limit are reported in the following table:

	Reproducibility limits R (log CFU/ml)		**SR_{alt}/ SR_{ref}**	**Conclusion**
	Reference method	**Alternative method**		
Level 1	0.272	0.539	1.98	Equivalence
Level 2	0.136	0.220	1.62	Equivalence
Level 3	0.230	0.400	1.74	Equivalence

Standard deviation reports of reproducibility are included between 1.62 and 1.98. At all three levels, they are larger than 0.5 and smaller than 2, there is no significant difference in the precision under reproducibility conditions between the alternative method and the reference method.

3.4.6 Conclusion

Fidelity of alternative method in repeatability conditions is biased with respect to the reference method at the L1 and L3 levels. And the precision under conditions of repeatability of both methods is considered to be equal at the L2 medium level.
The precision under conditions of repeatability and reproducibility of both methods is considered to be equal and is not dependant on the levels of microorganisms.

4 Practicability (STX vs RPFA)

Practicability is assessed according to criteria which are defined by the AFNOR Technical Committee. The 3M™ Petrifilm™ Staph Express system is compared to reference method NF ISO 6888-2 in terms of 13 criteria.
They are informed below:

Criterion	Communication on the criterion
1. Packaging 2. Reagents volumes	In **sealed pouches**. Petrifilm™ tests : packages of 2 x 25 units or 20 x 25 units. Disks: packages of 1 x 20 units or 5 x 20 units.
3. Storage conditions – Expiration date of unopened tests	Store unopened Petrifilm™ plates and disks pouches refrigerated or frozen at **temperature less than or equal to 8°C** . Expiration date is noted on each package of Petrifilm™plates and disks. (Period of validity of 18 month after the plant leaving).
4. Utilization procedure after first utilization	**Petrifilm™**: Return unused plates to pouch. Seal by folding the end of the pouch over and taping shut. To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches **in a cool dry place for no longer than one month.** Petrifilm™ Staph Express **disks** are individually packaged within a foil pouch. They are sensitive to both moisture and light. Remove only those individually packaged disks that will be used immediately; store remaining disks in the foil pouch by folding the end of the pouch and taping it shut. Place the resealed disk pouch in a sealable container and store **in a freezer for no longer than six months.** These informations are indicated in the 3M™ Petrifilm™ Staph Express Count System instructions.
5. Specific necessary equipment and premises	Usual configuration and equipment of a microbiological laboratory. Nothing specific except the **Petrifilm™ Flat Spreader** available at 3M. Easier reading with magnifying glass utilization.
6. Ready for use reagents or to restore	The Petrifilm™ system is **ready for use**.
7. Duration of training for a non initiated operator	For a laboratory technician fully formed to standard microbiological techniques, technique training requires **less than one day**.
8. Real time handling and technique flexibility in comparison with number of samples to analyze	**Saving of time** of 8 minutes maximum per positive sample in comparison with standard method. The time of handling is the same for a great or a small series of samples.
9. Response lead time	Result is obtained in **24 hours (D1**) whatever the test response: presence or not of coagulase-positive *Staphylococci* in the sample. The analysis of 1 sample according to standard EN ISO 6888-2 gives a result after 24 to 48 hrs (D2) for a sample.
10. Operator qualification type	The user must be trained to good laboratory practices (indicated in the STX instructions).
11. Joint stages with standard method	Mother suspension preparation, grinding and dilutions.
12. Analysis results traceability	**Lot number** is noted on each package of STX Petrifilm™ system. The lot number is also noted on individual plates and on the individual disk packages.
13. Laboratory maintenance	No particular service.

5 General conclusion

The validation study of 3M™ Petrifilm™ Staph Express (STX) method was conducted according to the reference document EN ISO 16 140 (2003).

The 3M™ Petrifilm™ Staph Express system for coagulase-positive *Staphylococcus* enumeration is a **miniaturized test** with a chromogenic medium selective and differential for coagulase-positive *Staphylococcus*, which doesn't need complementary confirmation tests. So, the Petrifilm Staph Express system is an **easy to use** method. And it allows a **saving of space in the incubators**.

Some reading difficulties can happen when products are contaminated with important levels of associated flora as for standard method.
The Petrifilm™ Staph Express system for coagulase-positive *Staphylococcus* enumeration allows a **saving of time** with regard to standard method, in particular for positive samples (result at Day 1 with regard to Day 1- Day 2 for standard method).

The **comparison** of Petrifilm™ Staph Express system with EN ISO 6888-2 standard allows concluding that the alternative method gives **accurate results** with regard to standard method.

The **linearity** of the alternative method is satisfactory.

The method is coagulase-positive *Staphylococcus* **specific** as for standard method.

Statistical interpretations of collaborative study have been calculated according to EN ISO 16140: 2003 and amendment 1, 2010 (NF EN ISO 16140/A1).

Collaborative study (accuracy) gave **satisfactory values of repeatability and of reproducibility.**

The alternative method and the reference method repeatability limits were statistically comparable for medium level (L2) and lower for low (L1) and high (L3) levels.

The precision under conditions of repeatability and reproducibility of both methods is considered to be equal and is not dependant on the levels of microorganisms.

For the alternative method, the **robust analysis** showed a **satisfactory interlaboratory coherence**, slightly better than the reference method.
Intralaboratory results were **comparable and less satisfactory for both methods**.

For all the contamination levels, the alternative method accuracy, relative to the reference method is satisfactory.
The bias values (alternative – reference) were similar to those obtained in the comparative study was (– 0.10/-0.15 log(CFU/ml or g)).

Set of results led to **NF validation renewal** according to EN ISO 16140, of the **3M™ Petrifilm™ Staph Express system (STX)** (certificate n° 3M 01/09-04/03 B), for the enumeration of coagulase-positive *Staphylococcus* in food products and pet food, **for a 4 years period**.

Lille, May 10th 2011

Melinda Maux
Technical manager

APPENDICES

APPENDIX A

RELATIVE ACCURACY
-
Detailed results for each sample category (CFU/g and log(CFU/g)) – synthesis

Synthesis

			EN ISO 6888-2 N (CFU/g)		Petrifilm staph Express N (CFU/g)		EN ISO 6888-2 log (N (CFU/g))		Petrifilm staph Express log (N (CFU/g))	
Code	Product	Cat	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
M2	Sausage	MP	<10	<10	<10	<10				
M11	Sirloin	MP	30	64	10	10	1,48	1,80	1,00	1,00
A1	Cooked andouillette	MP	<10	<10	<10	<10				
A4	Boiled ham	MP	105	123	>10 et <100	>10 et <100	2,02	2,09		
A5	Chicken fillet	MP	35	20	10	20	1,54	1,30	1,00	1,30
A6	Duck	MP	300	250	173	191	2,48	2,40	2,24	2,28
A11	Garlic sausage	MP	<10	<10	<10	<10				
A15	Beef	MP	10	<10	<10	<10	1,00			
A16	Chicken liveres	MP	>10 et <100	>10 et <100	<10	<10				
B6	Kidneys	MP	700	800	500	600	2,85	2,90	2,70	2,78
B7	Sausage meat	MP	291	359	373	345	2,46	2,56	2,57	2,54
B10	Chipolatas in herbs	MP	<10	<10	<10	<10				
B11	Toulouse sausage	MP	100	50	109	118	2,00	1,70	2,04	2,07
D6	Paprika chicken wings	MP	414	436	100	400	2,62	2,64	2,00	2,60
D12	Andouilettes	MP	3136	2773	1891	3300	3,50	3,44	3,28	3,52
D14	Minced meat	MP	1400	750	1400	1200	3,15	2,88	3,15	3,08
D15	Fricadelle (sausage)	MP	1409	2182	1700	1864	3,15	3,34	3,23	3,27
D16	Rissolettes breaded pork	MP	750	936	1455	800	2,88	2,97	3,16	2,90
3M06	Pork kidneys	MP	<10	<10	<10	<10				
3M15	Pork kidneys	MP	1455	864	991	882	3,16	2,94	3,00	2,95
3M17	Pork tongue	MP	<10	<10	100	100			2,00	2,00
3M22	Pork tongue	MP	>100 et <1000	>100 et <1000	1273	1382			3,10	3,14
3M23	Fresh breast	MP	<100	<100	90	109			1,95	2,04
3M40	Cured ham	MP	<10	<10	<100	<100				
3M41	Lasagna bolognese	MP	241	341	173	155	2,38	2,53	2,24	2,19
3M42	Lasagna bolognese	MP	745	755	336	264	2,87	2,88	2,53	2,42
3M53	Smoked lardons	MP	<10	<10	<10	<10				
3M54	Chorizo	MP	<10	<10	<10	<10				
3M57	Meat powder	MP	20000	18500	10000	20000	4,30	4,27	4,00	4,30
3M58	Meat powder	MP	31000	17000	10000	23000	4,49	4,23	4,00	4,36
3M59	Meat powder	MP	16500	17000	16000	15000	4,22	4,23	4,20	4,18
3M60	Bacon	MP	<10	<10	<10	<10				
3M61	Bacon	MP	<10	<10	<10	<10				
3M80	Minced meat	MP	<10	<10	<10	<10				
3M81	Sausage	MP	<10	<10	<10	<10				
3M82	Merguez	MP	<100	<100	<10	<10				
3M93	Ham	MP	<1000	<1000	<100	<100				
3M94	Giblets	MP	<1000	<1000	<10	<10				
3M95	Horse steak	MP	<1000	<1000	<10	<10				
L11	Ham	MP	>10 et <100	>10 et <100	10	30			1,00	1,48
L12	Quail thighs	MP	<10	<10	<10	<10				
L13	Turkey tenderloin	MP	<100	>10 et <100	30	30			1,48	1,48
L14	Sausage cooking smoke	MP	ILL	ILL	38000	36000			4,58	4,56
R1	Minced beefsteak	MP	<10	<10	<10	<10				
R2	Beef patty	MP	1750	1750	1555	1300	3,24	3,24	3,19	3,11
R3	Minced beefsteak	MP	305	350	473	527	2,48	2,54	2,67	2,72
R4	Minced beefsteak	MP	355	300	291	427	2,55	2,48	2,46	2,63
R8	Veal cutlet	MP	691	823	500	200	2,84	2,92	2,70	2,30
R13	Beef patty	MP	1500	1350	1645	1482	3,18	3,13	3,22	3,17
R14	Minced meat	MP	15	<10	<10	10	1,18			
R17	Veal cutlet	MP	40	55	70	20	1,60	1,74	1,85	1,30
R24	Rillette	MP	850	650	909	864	2,93	2,81	2,96	2,94
R25	Veal cutlet	MP	ILL	ILL	300	309			2,48	2,49
R26	Veal cutlet	MP	ILL	ILL	155	100			2,19	2,00
2007 CC3	Veal cutlet	MP	<10	<10	80	100			1,90	2,00

Synthesis

			EN ISO 6888-2 N (CFU/g)		Petrifilm staph Express N (CFU/g)		EN ISO 6888-2 log (N (CFU/g))		Petrifilm staph Express log (N (CFU/g))	
Code	Product	Cat	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
M1	Icecream	DP	350	391	255	191	2,54	2,59	2,41	2,28
M3	Raw milk	DP	<10	<10	<10	<10				
A3	Munster farmer cheese	DP	ILL	ILL	ILL	ILL				
A8	Frozen yogurt with strawberries	DP	114	80	90	30	2,06	1,90	1,95	1,48
A9	Frozen yogurt with peach	DP	55	82	80	30	1,74	1,91	1,90	1,48
A10	Vanilla frozen yogurt	DP	109	75	30	30	2,04	1,88	1,48	1,48
E7	Mimolette	DP	50	>10 et <100	<100	<100	1,70			
E8	Raclette cheese	DP	75	45	<10	<10	1,88	1,65		
E9	Gouda	DP	>10 et <100	>10 et <100	<10	<10				
E10	Gouda	DP	100	150	40	40	2,00	2,18	1,60	1,60
3M01	Reblochon	DP	500	<1000	ILL	ILL	2,70			
3M02	Reblochon	DP	ILL	ILL	ILL	ILL				
3M03	Reblochon	DP	1273	1136	ILL	ILL	3,10	3,06		
3M04	Reblochon	DP	ILL	ILL	ILL	ILL				
3M12	Munster farmer cheese	DP	ILL	ILL	ILL	ILL				
3M20	Gorgonzola	DP	<100	<100	<10	<10				
3M21	Munster farmer cheese	DP	19000	29000	<100	<100	4,28	4,46		
3M24	Reblochon	DP	ILL	ILL	1909	1300			3,28	3,11
3M25	Reblochon	DP	ILL	ILL	1909	2273			3,28	3,36
3M26	Reblochon	DP	ILL	ILL	<100	200				
3M27	Reblochon	DP	1182	1182	1273	1364	3,07	3,07	3,10	3,13
3M30	Ewe cheese	DP	11500	9500	>100 et <1000	>100 et <1000	4,06	3,98		
3M31	Morbier	DP	<100	<100	<10	<10				
3M36	Goat cheese	DP	<100	<100	100	100			2,00	2,00
3M38	Goat cheese	DP	200	500	100	>10 et <100	2,30	2,70		
L1	Raw milk	DP	40	45	10	<10	1,60	1,65	1,00	
L2	Raw milk	DP	186	118	100	127	2,27	2,07	2,00	2,10
L3	Raw cream	DP	323	391	255	418	2,51	2,59	2,41	2,62
L4	Raw cream	DP	114	145	173	100	2,06	2,16	2,24	2,00
L5	Raw cream	DP	150	100	200	200	2,18	2,00	2,30	2,30
L6	Goat farmer cheese	DP	3636	3182	300	500	3,56	3,50	2,48	2,70
L7	Goat farmer cheese	DP	168	114	<100	<10	2,23	2,06		
L8	Goat raw milk	DP	70	60	70	50	1,85	1,78	1,85	1,70
L9	Raw milk	DP	50	50	50	60	1,70	1,70	1,70	1,78
L10	Raw milk	DP	250	250	>10 et <100	>10 et <100	2,40	2,40		
L15	Farmer cheese	DP	714	923	100	90	2,85	2,97	2,00	1,95
L16	Farmer cheese	DP	1100	>100 et <1000	<100	<100	3,04			
R20	Goat cheese	DP	ILL	ILL	336	355			2,53	2,55
R21	Goat cheese	DP	ILL	ILL	60	60			1,78	1,78
R30	Saint Nectaire cheese	DP	8636	10000	14727	18455	3,94	4,00	4,17	4,27
R32	Raw milk	DP	218	345	273	209	2,34	2,54	2,44	2,32
2007 D1	Tartine tilt	DP	63636	70909	40000	48182	4,80	4,85	4,60	4,68
2007 D2	Raclette cheese	DP	10000	12727	22727	32727	4,00	4,10	4,36	4,51
2007 AA5	Savoie cheese	DP	3500	3727	1364	1455	3,54	3,57	3,13	3,16
2007 B2	Raw milk	DP	10	<10	<10	10	1,00			1,00
2007 B3	Epoisses cheese	DP	<10	<10	<10	<10				
2007 B4	St Priest cheese	DP	<10	<10	<10	<10				
2007 C1	Raw milk	DP	<10	<10	<10	<10				
2007 Y3	Strawberry icecream	DP	40	80	20	30	1,60	1,90	1,30	1,48

Synthesis

			EN ISO 6888-2 N (CFU/g)		Petrifilm staph Express N (CFU/g)		EN ISO 6888-2 log (N (CFU/g))		Petrifilm staph Express log (N (CFU/g))	
Code	Product	Cat	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
M6	Net of sole	SF	ILL	ILL	20	10			1,30	1,00
A13	Surimi salad	SF	>100 et<1000	>100 et<1000	ILL	>100 et<1000				
C14	Sardine	SF	ILL	ILL	ILL	>1000				
C15	Smoked salmon	SF	ILL	ILL	900	1000			2,95	3,00
C17	Salmon in the dill	SF	ILL	ILL	<100	<100				
C19	Baked salmon	SF	15	25	10	10	1,18	1,40	1,00	1,00
C20	Surimi	SF	618	682	891	1164	2,79	2,83	2,95	3,07
C21	Plate marinades	SF	ILL	ILL	ILL	ILL				
D8	Net of trout	SF	10	45	100	100	1,00	1,65	2,00	2,00
3M05	Surimi	SF	<10	<10	<10	<10				
3M18	Shrimps	SF	>10 et <100	<10	<10	<10				
3M32	Net of tropical sole	SF	<1000	<1000	<10	<10				
3M35	Lasagna salmon	SF	7727	9091	6091	4182	3,89	3,96	3,78	3,62
3M43	Lasagna salmon	SF	3000	3864	2909	2182	3,48	3,59	3,46	3,34
3M44	Smoked salmon	SF	<10	<10	<10	<10				
3M51	Shrimps salad	SF	10	30	50	30	1,00	1,48	1,70	1,48
3M52	Terrine of scampis	SF	ILL	ILL	<10	<10				
R9	Shrimps	SF	<10	<10	<100	<100				
R10	Shrimps	SF	205	195	145	200	2,31	2,29	2,16	2,30
R11	Shrimps	SF	70	60	60	60	1,85	1,78	1,78	1,78
R12	Shrimps	SF	25	45	80	100	1,40	1,65	1,90	2,00
R27	Piece of cod	SF	25	55	10	60	1,40	1,74	1,00	1,78
R28	Fillet of sea bass	SF	40	50	50	60	1,60	1,70	1,70	1,78
R34	Shrimps - surimi	SF	3000	2500	2636	2182	3,48	3,40	3,42	3,34
R37	Cooked fish	SF	327	309	327	318	2,51	2,49	2,51	2,50
R38	Whiting fillet	SF	300	318	336	209	2,48	2,50	2,53	2,32
R39	Carpaccio of salmon	SF	232	123	218	155	2,37	2,09	2,34	2,19
2007 AA4	Cocktail of shrimps	SF	<10	<10	<10	<10				
2007 AA6	Net of hake	SF	<10	<10	<10	<10				
2007 CC2	Net of cod	SF	<10	<10	<10	<10				
2007 A1	Net Emperor	SF	35	30	40	10	1,54	1,48	1,60	1,00
A2	Salad	V	73	30	100	>10 et <100	1,86	1,48	2,00	
A12	Salad(corn, pepper, artichoke)	V	<10	<10	<10	<10				
A14	Salad (white cabbage/ham)	V	86	127	30	50	1,94	2,10	1,48	1,70
D7	Celery remoulade	V	1182	2045	1227	818	3,07	3,31	3,09	2,91
D19	Salad (carrots celery)	V	359	295	182	245	2,56	2,47	2,26	2,39
3M08	Vegetables for couscous	V	<10	<10	<10	<10				
3M14	Salad(potatoes,ham,tomatoes)	V	<10	<10	<10	<10				
3M16	Cabbage	V	<100	<100	<100	<100				
3M28	Deep-frozen vegetables	V	155	164	109	182	2,19	2,21	2,04	2,26
3M29	Powder with cocoa	V	<100	<100	<100	<100				
3M37	Salad (rice, peppers, olives)	V	ILL	ILL	<10	<10				
3M39	Sauerkraut	V	114	105	127	109	2,06	2,02	2,10	2,04
3M62	Salad	V	<10	<10	<10	<10				
R5	Salade (potatoes, tuna)	V	<10	<10	<10	<10				
R6	Mixed vegetables	V	<10	<10	<10	<10				
R7	Salad (shredded cabbage)	V	<10	<10	<10	<10				
R15	Tabbouleh	V	25	15	10	10	1,40	1,18	1,00	1,00
R33	Mixed salad	V	368	423	436	382	2,57	2,63	2,64	2,58
R35	Celery remoulade	V	4591	4773	2636	3545	3,66	3,68	3,42	3,55
R36	Carrots - tomatoes	V	5045	4409	3636	3818	3,70	3,64	3,56	3,58
2007 A2	Pasta salad	V	25	15	10	10	1,40	1,18	1,00	1,00
2007 DD2	Salad	V	9182	9727	7909	8273	3,96	3,99	3,90	3,92
2007 F1	Chopped salad	V	359091	322727	148182	135455	5,56	5,51	5,17	5,13

Synthesis

			EN ISO 6888-2 N (CFU/g)		Petrifilm staph Express N (CFU/g)		EN ISO 6888-2 log (N (CFU/g))		Petrifilm staph Express log (N (CFU/g))	
Code	Product	Cat	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
M4	Pastry	Div	105	127	145	127	2,02	2,10	2,16	2,10
M5	Pastry	Div	6591	21000	7727	10545	3,82	4,32	3,89	4,02
M7	Pastry	Div	50500	37000	30000	18455	4,70	4,57	4,48	4,27
A7	Pastry (Princess Summer)	Div	10500	12500	8000	9182	4,02	4,10	3,90	3,96
B3	Pastry (Square cream)	Div	ILL	ILL	10500	12300			4,02	4,09
B5	Pastry (Paris Brest)	Div	7455	6200	5100	5600	3,87	3,79	3,71	3,75
B12	Pastry(Paris vanilla pastry)	Div	818	955	173	164	2,91	2,98	2,24	2,21
B13	Pastry (merveilleux chocolate)	Div	5091	6045	1227	1100	3,71	3,78	3,09	3,04
C24	Pastry (peach Melba)	Div	2450	2150	1182	973	3,39	3,33	3,07	2,99
D10	Boiled egg mayonnaise	Div	300	300	436	591	2,48	2,48	2,64	2,77
3M07	Pastry	Div	<1000	<1000	<10	10				
3M09	Liquide whole egg	Div	<1	<1	1	1			0,00	0,00
3M10	Liquide whole egg	Div	<1	<1	<1	<1				
3M11	Pastry (creme caramel)	Div	<10	<10	<10	<10				
3M13	Pastry	Div	<10	<10	<10	<10				
3M19	Pastry (coffee eclair)	Div	1091	1583	1182	1182	3,04	3,20	3,07	3,07
2007 CC1	Custard	Div	<10	<10	<10	<10				
2007 Z1	Dog food	AN	20455	25000	17273	16364	4,31	4,40	4,24	4,21
2007 Z2	Waste meat feed	AN	20909	21818	14545	17273	4,32	4,34	4,16	4,24
2007 B1	Sausage feed	AN	<10	<10	<10	<10				
2007 C2	Granules for animals	AN	<10	<10	<10	<10				
2007 BB3	Granules for animals	AN	<10	<10	<10	<10				
2007 BB4	Granules for animals	AN	<10	<10	<10	<10				
2007 AA1	Dog food	AN	<10	<10	<10	<10				
2007 AA2	Cat food	AN	<10	<10	<10	<10				
2007 AA3	Cat food	AN	<10	<10	<10	<10				
2007 AA4	Powder of meat	AN	<10	<10	<10	<10				
2007 Y1	Pâté lamb / vegetables	AN	30	40	40	10	1,48	1,60	1,60	1,00
2007 Y2	Pâté lamb / vegetables	AN	75	155	10	<10	1,88	2,19	1,00	
2007 X1	Cat food	AN	336	355	100	70	2,53	2,55	2,00	1,85
2007 X2	Cat food	AN	4318	4818	764	855	3,64	3,68	2,88	2,93
2007 X3	Fish meal	AN	30	15	50	40	1,48	1,18	1,70	1,60
2007 W1	Waste meat feed	AN	559	627	336	336	2,75	2,80	2,53	2,53
2007 W2	Waste meat feed	AN	6500	7091	2182	2182	3,81	3,85	3,34	3,34
2007 W3	Waste meat feed	AN	5318	4909	1818	1909	3,73	3,69	3,26	3,28
2007 E1	Granules for animals	AN	409091	450000	120909	137273	5,61	5,65	5,08	5,14
2007 E2	Feed grains	AN	26364	22273	13273	12909	4,42	4,35	4,12	4,11
2007 E3	Cat food	AN	2545	1955	1091	1182	3,41	3,29	3,04	3,07
2007 E4	Fresh meat for dog	AN	20455	16364	7727	7636	4,31	4,21	3,89	3,88

Cat : category of products
MP : Meat Products
DP : Dairy Products
SF : Seafood
V : Vegetables
D : Diverse food products
AN : Animal feed
Rep1 - Rep 2 : replicate 1 - replicate 2

APPENDIX B

RELATIVE ACCURACY

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Results of accuracy regression for all products

All products

		Reference method				Alternative method			
	Level	Rep.1	Rep.2	Mxi	sxi	Rep.1	Rep.2	Myi	syi
Meat products	1	1,48	1,80	1,64041	0,23093	1,00	1,00	1,00000	0,00000
	2	1,54	1,30	1,42255	0,17185	1,00	1,30	1,15051	0,21286
	3	2,48	2,40	2,43753	0,05599	2,24	2,28	2,25909	0,03073
	4	2,85	2,90	2,87409	0,04101	2,70	2,78	2,73856	0,05599
	5	2,46	2,56	2,50948	0,06466	2,57	2,54	2,55489	0,02333
	6	2,00	1,70	1,84949	0,21286	2,04	2,07	2,05517	0,02458
	7	2,62	2,64	2,62823	0,01643	2,00	2,60	2,30103	0,42572
	8	3,50	3,44	3,46967	0,03784	3,28	3,52	3,39759	0,17101
	9	3,15	2,88	3,01059	0,19167	3,15	3,08	3,11265	0,04734
	10	3,15	3,34	3,24388	0,13427	3,23	3,27	3,25041	0,02822
	11	2,88	2,97	2,92325	0,06815	3,16	2,90	3,03291	0,18359
	12	3,16	2,94	3,04953	0,16009	3,00	2,95	2,97071	0,03582
	13	2,38	2,53	2,45725	0,10662	2,24	2,19	2,21321	0,03416
	14	2,87	2,88	2,87505	0,00372	2,53	2,42	2,47391	0,07481
	15	4,30	4,27	4,28410	0,02394	4,00	4,30	4,15051	0,21286
	16	4,49	4,23	4,36091	0,18449	4,00	4,36	4,18086	0,25578
	17	4,22	4,23	4,22397	0,00917	4,20	4,18	4,19011	0,01982
	18	3,24	3,24	3,24304	0,00000	3,19	3,11	3,15277	0,05491
	19	2,48	2,54	2,51386	0,04272	2,67	2,72	2,69832	0,03353
	20	2,55	2,48	2,51340	0,05130	2,46	2,63	2,54723	0,11805
	21	2,84	2,92	2,87734	0,05362	2,70	2,30	2,50000	0,28139
	22	3,18	3,13	3,15321	0,03236	3,22	3,17	3,19354	0,03217
	23	1,60	1,74	1,67121	0,09779	1,85	1,30	1,57306	0,38471
	24	2,93	2,81	2,87117	0,08238	2,96	2,94	2,94747	0,01575
Dairy products	25	2,54	2,59	2,56807	0,03395	2,41	2,28	2,34330	0,08835
	26	2,06	2,12	2,08775	0,04558	1,95	1,48	1,71568	0,33738
	27	1,74	1,91	1,82480	0,12452	1,90	1,48	1,69011	0,30121
	28	2,04	1,88	1,95642	0,11507	1,48	1,48	1,47712	0,00000
	29	2,00	2,18	2,08805	0,12452	1,60	1,60	1,60206	0,00000
	30	3,07	3,07	3,07255	0,00000	3,10	3,11	3,10934	0,00651
	31	2,27	2,07	2,17146	0,13987	2,00	2,10	2,05237	0,07406
	32	2,51	2,59	2,55046	0,05886	2,41	2,62	2,51357	0,15245
	33	2,06	2,16	2,11036	0,07406	2,24	2,00	2,11868	0,16784
	34	2,18	2,00	2,08805	0,12452	2,30	2,30	2,30103	0,00000
	35	3,56	3,50	3,53167	0,04101	2,48	2,70	2,58805	0,15687
	36	1,85	1,78	1,81162	0,04734	1,85	1,70	1,77203	0,10333
	37	1,70	1,70	1,69897	0,00000	1,70	1,78	1,73856	0,05599
	38	2,85	2,97	2,90928	0,07891	2,00	1,95	1,97712	0,03236
	39	3,94	4,00	3,96817	0,04502	4,17	4,27	4,21711	0,06928
	40	2,34	2,54	2,43860	0,14112	2,44	2,32	2,37803	0,08160
	41	4,80	4,85	4,82720	0,03323	4,60	4,68	4,64247	0,05715
	42	4,00	4,10	4,05237	0,07406	4,36	4,51	4,43573	0,11198
	43	3,54	3,57	3,55773	0,01932	3,13	3,16	3,14871	0,01982
	44	1,60	1,90	1,75257	0,21286	1,30	1,48	1,38908	0,12452
Seafood products	45	1,18	1,40	1,28702	0,15687	1,00	1,00	1,00000	0,00000
	46	2,79	2,83	2,81239	0,03009	2,95	3,07	3,00783	0,08201
	47	1,00	1,65	1,32661	0,46189	2,00	2,00	2,00000	0,00000
	48	3,89	3,96	3,92332	0,04991	3,78	3,62	3,70302	0,11548
	49	3,48	3,59	3,53206	0,07769	3,46	3,34	3,40129	0,08835
	50	1,00	1,48	1,23856	0,33738	1,70	1,48	1,58805	0,15687
	51	2,31	2,29	2,30092	0,01396	2,16	2,30	2,23188	0,09779
	52	1,85	1,78	1,81162	0,04734	1,78	1,78	1,77815	0,00000
	53	1,40	1,65	1,52558	0,18050	1,90	2,00	1,95154	0,06853
	54	1,40	1,74	1,56915	0,24213	1,00	1,78	1,38908	0,55024
	55	1,60	1,70	1,65051	0,06853	1,70	1,78	1,73856	0,05599
	56	3,48	3,40	3,43753	0,05599	3,42	3,34	3,37991	0,05811
	57	2,51	2,49	2,50250	0,01755	2,51	2,50	2,50879	0,00865
	58	2,48	2,50	2,48990	0,01807	2,53	2,32	2,42357	0,14600
	59	2,37	2,09	2,22704	0,19531	2,34	2,19	2,26394	0,10590
	60	1,54	1,48	1,51059	0,04734	1,60	1,00	1,30103	0,42572
Vegetables	61	1,94	2,10	2,02053	0,11908	1,48	1,70	1,58805	0,15687
	62	3,07	3,31	3,19167	0,16846	3,09	2,91	3,00090	0,12452
	63	2,56	2,47	2,51285	0,05990	2,26	2,39	2,32480	0,09216
	64	2,19	2,21	2,20147	0,01755	2,04	2,26	2,14871	0,15687
	65	2,06	2,02	2,03741	0,02561	2,10	2,04	2,07126	0,04734
	66	1,40	1,18	1,28702	0,15687	1,00	1,00	1,00000	0,00000
	67	2,57	2,63	2,59606	0,04242	2,64	2,58	2,61085	0,04101
	68	3,66	3,68	3,67033	0,01193	3,42	3,55	3,48534	0,09098
	69	3,70	3,64	3,67362	0,04140	3,56	3,58	3,57126	0,01498
	70	1,40	1,18	1,28702	0,15687	1,00	1,00	1,00000	0,00000
	71	3,96	3,99	3,97546	0,01772	3,90	3,92	3,90789	0,01380
	72	5,56	5,51	5,53202	0,03279	5,17	5,13	5,15129	0,02758
Pastries	73	2,02	2,10	2,06202	0,06041	2,16	2,10	2,13373	0,04101
	74	3,82	4,32	4,07058	0,35587	3,89	4,02	3,95555	0,09549
	75	4,70	4,57	4,63575	0,09552	4,48	4,27	4,37161	0,14921
	76	4,02	4,10	4,05905	0,05354	3,90	3,96	3,93301	0,04231
	77	3,80	3,78	3,79093	0,01807	3,71	3,75	3,72788	0,02872
	78	2,91	2,98	2,94632	0,04734	2,24	2,21	2,22562	0,01660
	79	3,71	3,78	3,74411	0,05277	3,09	3,04	3,06517	0,03362
	80	3,39	3,33	3,36080	0,04011	3,07	2,99	3,03027	0,05979
	81	2,48	2,48	2,47712	0,00000	2,64	2,77	2,70568	0,09311
	82	3,04	3,20	3,11868	0,11440	3,07	3,07	3,07255	0,00000
Pet foods	83	4,31	4,40	4,35436	0,06162	4,24	4,21	4,22562	0,01660
	84	4,32	4,34	4,32958	0,01307	4,16	4,24	4,20004	0,05277
	85	1,48	1,60	1,53959	0,08835	1,60	1,00	1,30103	0,42572
	86	2,53	2,55	2,53824	0,01617	2,00	1,85	1,92255	0,10953
	87	3,64	3,68	3,65909	0,03365	2,88	2,93	2,90731	0,03454
	88	1,48	1,18	1,32661	0,21286	1,70	1,60	1,65051	0,06853
	89	2,75	2,80	2,77247	0,03534	2,53	2,53	2,52681	0,00000
	90	3,81	3,85	3,83181	0,02672	3,34	3,34	3,33882	0,00000
	91	3,73	3,69	3,70838	0,02458	3,26	3,28	3,27023	0,01498
	92	5,52	5,63	5,57580	0,07764	5,08	5,14	5,11002	0,03898
	93	4,47	4,46	4,46712	0,00476	4,12	4,11	4,11693	0,00853
	94	3,02	3,03	3,02678	0,00525	3,04	3,07	3,05517	0,02458
	95	4,06	3,97	4,01712	0,06162	3,89	3,88	3,88546	0,00363

Median x	2,77247	0,05362	
Mean x	2,83908		
Srx		0,11738	
Rob Swx		0,07950	
R		1,21925	
Rob.R		1,04413	

Median y	2,54723	0,05599	
Mean y	2,69593		
Sry		0,14312	
Rob Swy		0,08301	

n = 2
q = 95
nq = 190

All products

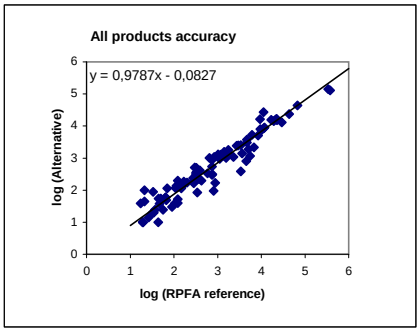
0,5 < R < 2	GMFR
R > 2	OLS
R < 0,5	OLS chgt

GMFR regression

Global standard deviations

Vxi	Vyi
2,92692	5,75235
4,04264	4,82192
0,32562	0,38259
0,00413	0,00677
0,22145	0,04033
2,00390	0,82175
0,08918	0,49313
0,79672	1,01391
0,09557	0,34956
0,34575	0,61568
0,01882	0,26082
0,11421	0,15229
0,30296	0,46720
0,00260	0,10418
4,17675	4,27695
4,66595	4,47549
3,83591	4,46552
0,32637	0,42043
0,21336	0,00114
0,21477	0,05816
0,00580	0,15595
0,19841	0,49627
2,73739	2,66966
0,00885	0,12679
0,14804	0,25650
1,13108	2,03559
2,07301	2,11409
1,57139	2,97098
1,14360	2,39310
0,10902	0,34186
0,91100	0,83383
0,17007	0,08975
1,06755	0,69460
1,14360	0,31189
0,96105	0,04789
2,11356	1,71784
2,59969	1,83624
0,01608	1,03442
2,55170	4,63280
0,34067	0,20877
7,90639	7,58132
2,94963	6,06635
1,03329	0,41042
2,40629	3,43123
4,84241	5,75235
0,00233	0,20128
4,78849	0,96863
2,35364	2,04182
0,96648	1,00287
5,23714	2,47942
0,57943	0,44025
2,11356	1,68463
3,48316	1,11291
3,28406	3,71849
2,83006	1,83624
0,71943	0,93904
0,22688	0,07011
0,24418	0,16967
0,78732	0,38445
3,53198	4,07272
1,35421	2,47942
0,27702	0,20151
0,21644	0,28396
0,81340	0,62350
1,28600	0,78266
4,84241	5,75235
0,11991	0,01616
1,38211	1,25461
1,39465	1,53264
4,84241	5,75235
2,58304	2,93788
14,50495	12,05840
1,21129	0,63381
3,15985	3,18239
6,46516	5,63809
2,97953	3,06253
1,81236	2,13067
0,02524	0,44266
1,64096	0,27380
0,54600	0,22714
0,26203	0,00886
0,16944	0,28369
4,59598	4,68019
4,44334	4,52751
3,38514	4,07272
0,18127	1,20823
1,34598	0,09056
4,62045	2,19048
0,01012	0,05720
1,97174	0,82661
1,51198	0,65987
14,98535	11,65721
5,30109	4,03855
0,07049	0,25871
2,77938	2,82996

Vx	Vy	Vxy
1,02170	0,97868	0,94846
Sx	Sy	
1,01079	0,98928	



Estimations

r =	0,96184
b =	0,97872
a =	-0,08273

All products

Residual standard deviation from the estimated points by regression

Sy:x =	0,387916365

yi estimated	residus	Smy:x
1,52277	-0,52277	0,27430
1,30955	-0,15903	
2,30293	-0,04383	
2,73020	0,00836	
2,37335	0,18154	
1,72740	0,32777	
2,48957	-0,18854	
3,31310	0,08450	
2,86379	0,24886	
3,09211	0,15829	
2,77831	0,25460	
2,90190	0,06881	
2,32222	-0,10901	
2,73114	-0,25723	
4,11020	0,04032	
4,18537	-0,00450	
4,05134	0,13876	
3,09129	0,06148	
2,37763	0,32069	
2,37718	0,17005	
2,73337	-0,23337	
3,00338	0,19016	
1,55292	0,02015	
2,72733	0,22014	
2,43069	-0,08739	
1,96059	-0,24491	
1,70324	-0,01314	
1,83206	-0,35494	
1,96088	-0,35882	
2,92443	0,18491	
2,04252	0,00985	
2,41345	0,10012	
1,98272	0,13596	
1,96088	0,34015	
3,37378	-0,78574	
1,69034	0,08169	
1,58008	0,15848	
2,76463	-0,78751	
3,80099	0,41613	
2,30398	0,07405	
4,64174	0,00073	
3,88340	0,55233	
3,39929	-0,25057	
1,63255	-0,24347	
1,17690	-0,17690	
2,66981	0,33801	
1,21565	0,78435	
3,75709	-0,05407	
3,37416	0,02713	
1,12947	0,45857	
2,16922	0,06266	
1,69034	0,08781	
1,41038	0,54116	
1,45303	-0,06395	
1,53266	0,20590	
3,28164	0,09827	
2,36651	0,14228	
2,35418	0,06939	
2,09692	0,16702	
1,39572	-0,09469	
1,89480	-0,30676	
3,04102	-0,04012	
2,37664	-0,05184	
2,07189	0,07682	
1,91132	0,15994	
1,17690	-0,17690	
2,45808	0,15277	
3,50949	-0,02415	
3,51271	0,05855	
1,17690	-0,17690	
3,80813	0,09976	
5,33156	-0,18026	
1,93541	0,19832	
3,90122	0,05432	
4,45436	-0,08275	
3,88994	0,04307	
3,62752	0,10036	
2,80089	-0,57527	
3,58170	-0,51653	
3,20655	-0,17628	
2,34168	0,36401	
2,96958	0,10297	
4,17897	0,04665	
4,15471	0,04534	
1,42410	-0,12307	
2,40149	-0,47894	
3,49849	-0,59118	
1,21565	0,43487	
2,63074	-0,10393	
3,66753	-0,32871	
3,54673	-0,27650	
5,37441	-0,26439	
4,28933	-0,17240	
2,87963	0,17554	
3,84890	0,03655	

Standard deviations of parameters

S(a)	0,08430	t(a)	0,98133	p(a=0)	0,32898
S(b)	0,02799	t(b)	0,76037	p(b=1)	0,44896

All products

Repetability
= 2,8 Sr

	Reference method	Alternative method
Sr	0,11738	0,14312
r	0,32866	0,40072
Rob.Sr	0,07950	0,08301
Rob.r	0,22261	0,23243

Bias

Differences
-0,64041
-0,27203
-0,17844
-0,13553
0,04541
0,20568
-0,32720
-0,07207
0,10206
0,00653
0,10966
-0,07882
-0,24404
-0,40115
-0,13359
-0,18004
-0,03386
-0,09026
0,18446
0,03383
-0,37734
0,04033
-0,09815
0,07630
-0,22478
-0,37206
-0,13470
-0,47930
-0,48599
0,03679
-0,11909
-0,03689
0,00832
0,21298
-0,94363
-0,03959
0,03959
-0,93215
0,24895
-0,06057
-0,18473
0,38336
-0,40902
-0,36350
-0,28702
0,19543
0,67339
-0,22029
-0,13077
0,34949
-0,06904
-0,03347
0,42597
-0,18008
0,08805
-0,05762
0,00629
-0,06633
0,03689
-0,20956
-0,43249
-0,19077
-0,18804
-0,05276
0,03385
-0,28702
0,01479
-0,18499
-0,10236
-0,28702
-0,06757
-0,38073
0,07171
-0,11504
-0,26413
-0,12604
-0,06305
-0,72070
-0,67895
-0,33053
0,22856
-0,04613
-0,12874
-0,12953
-0,23856
-0,61569
-0,75178
0,32391
-0,24566
-0,49299
-0,43815
-0,46578
-0,35020
0,02839
-0,13167

D =	-0,14315	mean
D =	-0,12604	median

APPENDIX C

RESULTS OF SPECIFICITY / SELECTIVITY

SPECIFICITY/SELECTIVITY

Strains	Origin	Results after 1st incubation	Results after disk insertion
Staphylococcus aureus	ATCC 6538	red-violet	pink zone
Characteristic colonies on Baird-Parker agar medium	ATCC 9144	red-violet	pink zone
	Dairy product	red-violet	pink zone
	Meat product	red-violet	pink zone
	Raw milk	red-violet	pink zone
	Raw milk cheese	red-violet	pink zone
	Dairy product	red-violet	pink zone
	Dairy product	red-violet	pink zone
	Raw milk cheese	red-violet	pink zone
	Raw milk cheese	red-violet	pink zone
	Chipolatas	red-violet	pink zone
	Meat product	red-violet	pink zone
	Meat product	red-violet	pink zone
	Meat product	red-violet	pink zone
	Meat product	red-violet	pink zone
	Meat product	red-violet	pink zone
	CIP 7625	red-violet	pink zone
	Cake	red-violet	pink zone
	Cake	red-violet	pink zone
	Smoked salmon	red-violet	pink zone
	Milk	red-violet	pink zone
	CIP 53154	red-violet	pink zone
	Fish filet	red-violet	pink zone
	Salad	red-violet	pink zone
	Toast	red-violet	pink zone
Staphylococcus aureus	Meat product	red-violet	pink zone
No characteristic colonies on Baird-Parker agar medium	Poultry liver	red-violet	pink zone
	Goat milk	red-violet	pink zone
St.hyicus	Collection	red-violet	pink zone
St.hyicus	Meat product	black	pink zone
St.hyicus	Meat product	black	pink zone
St.hyicus	Meat product	black	pink zone
St.hyicus	Collection	black	pink zone
St intermedius	Collection	red-violet	pink zone
St intermedius	Collection	violet	pink zone
St.xylosus	Munster (cheese)	black	no pink zone
St.epidermidis	Dairy product	no colonie	/
St.epidermidis	ATCC 12228	no colonie	/
St.scuiri	Collection	no colonie	/
St.saprophyticus	Collection	black	no pink zone
St.cohnii	Smoked salmon	no colonie	/
St.epidermidis	Clinical	no colonie	/
St.epidermidis	Smoked salmon	no colonie	/
St.epidermidis	Collection	no colonie	/
St.simulans	Salad	black	no pink zone
St.warneri	Ham	no colonie	/
St.warneri	Bacon	no colonie	/
St.warneri	Bayonne ham	no colonie	/
St.xylosus	Salad	black	no pink zone
St.xylosus	Offal	black	no pink zone
Other genus			
Listeria innocua	Smoked fish	blue	no pink zone
Enterococcus faecalis	Meat product	no colonie	/
Micrococcus spp	Vegetables	no colonie	/
E.coli	Dairy product	no colonie	/
Micrococcus spp	Environment	no colonie	/
Micrococcus luteus	Environment	no colonie	/
Micrococcus roseus	Environment	no colonie	/
Enterococcus faecalis	Eggs	blue-green	no pink zone
Enterococcus faecium	ATCC 3286	blue-green	no pink zone
Enterococcus faecium	CIP 5433	no colonie	/
Enterococcus durans	Meat product	no colonie	/

APPENDIX D

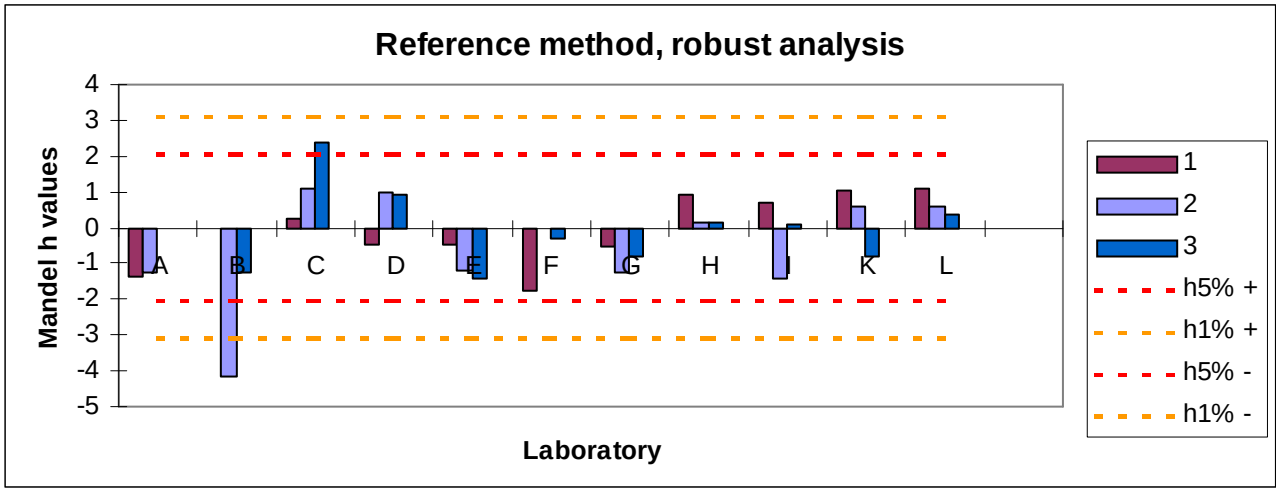
INTERLABORATORY STUDY

Control of the coherence of the results of measurement with *h* and *k* Mandel values

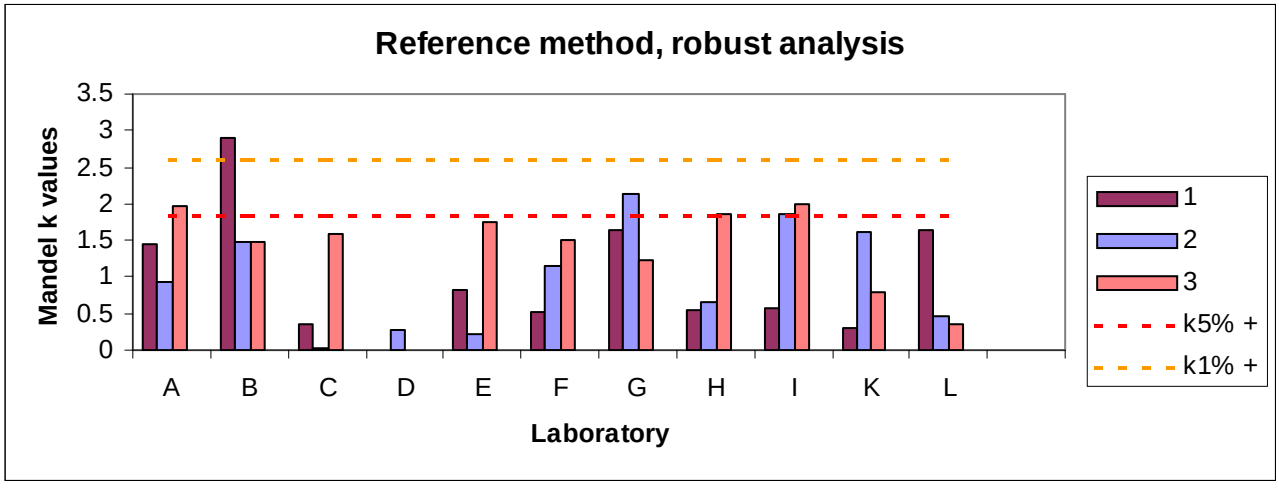
Graphs

Control of the coherence of the reference method

Mandel h_{ij} inter-laboratory statistical coherence

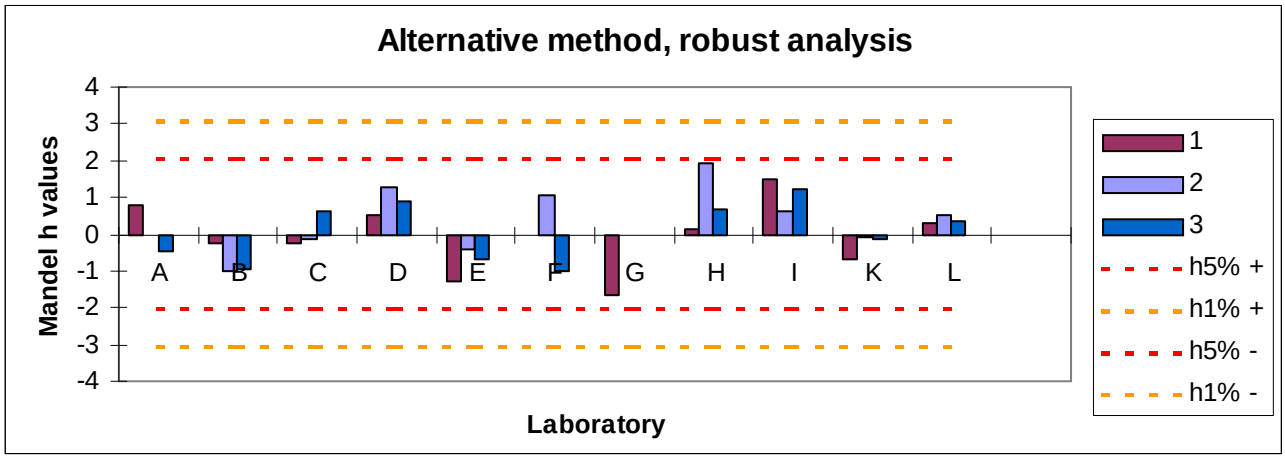


Mandel k_{ij} intra-laboratory statistical coherence



Control of the coherence of the alternative method

Mandel h_{ij} inter-laboratory statistical coherence



Mandel k_{ij} intra-laboratory statistical coherence