



AFNOR validation according to the ISO 16140
of the 3M™ Petrifilm™ Staph Express System
(STX system) for the
“coagulase positive Staphylococcus”
enumeration in food products

-
Reference method EN ISO 6888-2

Summary report

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1 Introduction

1.1 Validation references

The 3M™ Petrifilm™ Staph Express Count system (STX) has been validated according to the reference method EN ISO 16140:2003, with respect to the reference method ISO 6888-2.

1.2 Protocol and principle of the alternative method

1.2.1 Principle of the 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

Analytical diagrams are presented on figures 1 and 2.

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

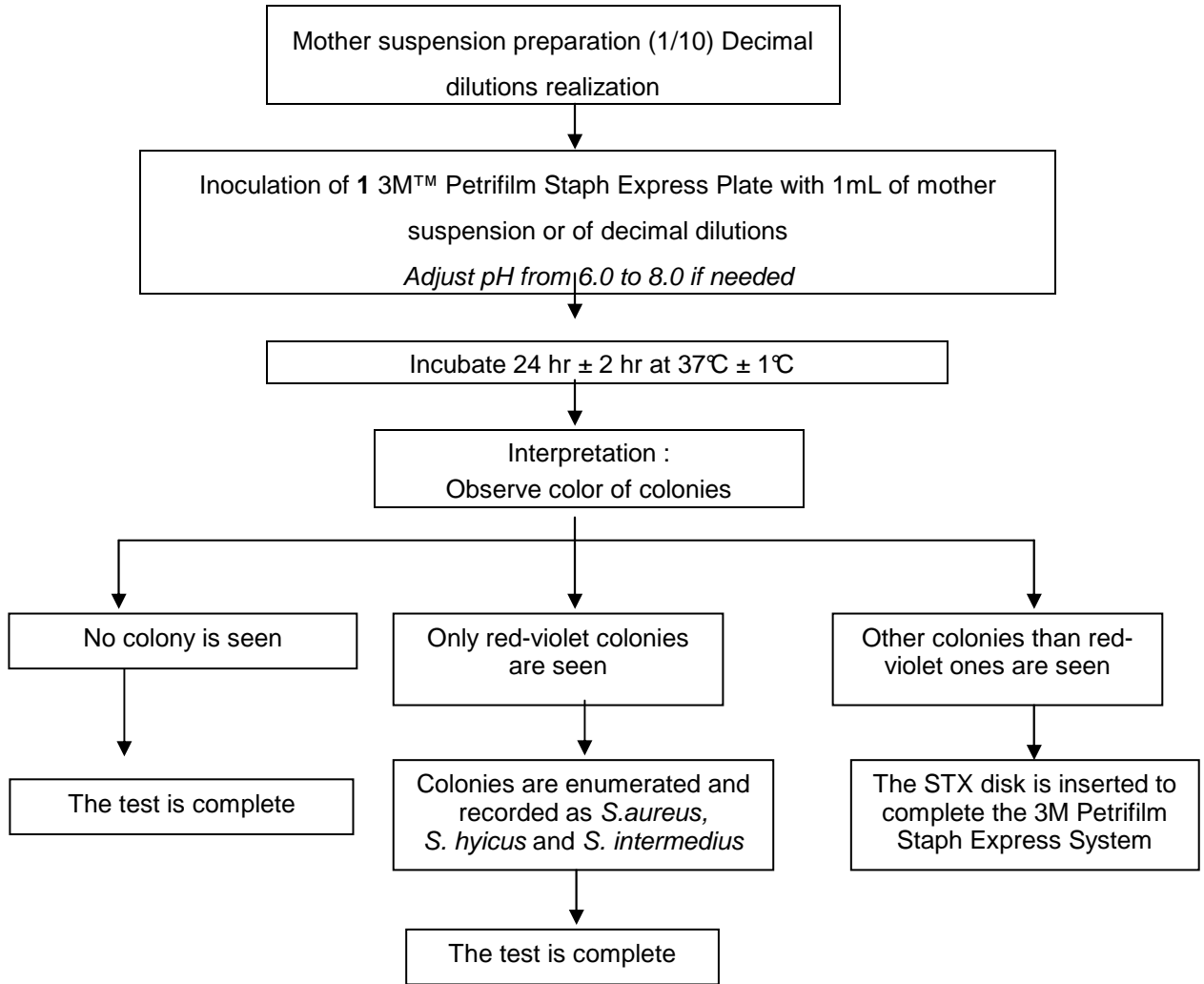
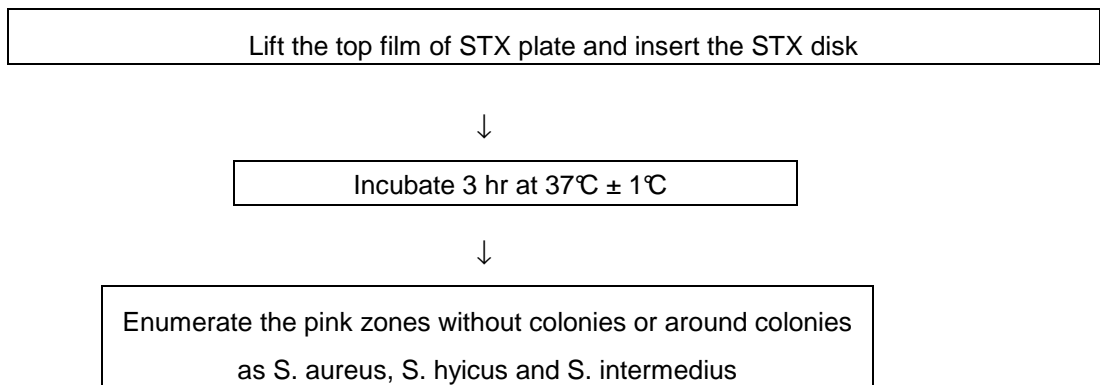


Figure 2 : 3M™ Petrifilm™ Staph Express Disk INSERTION



1.3 Scope

All human food products and pet foods.

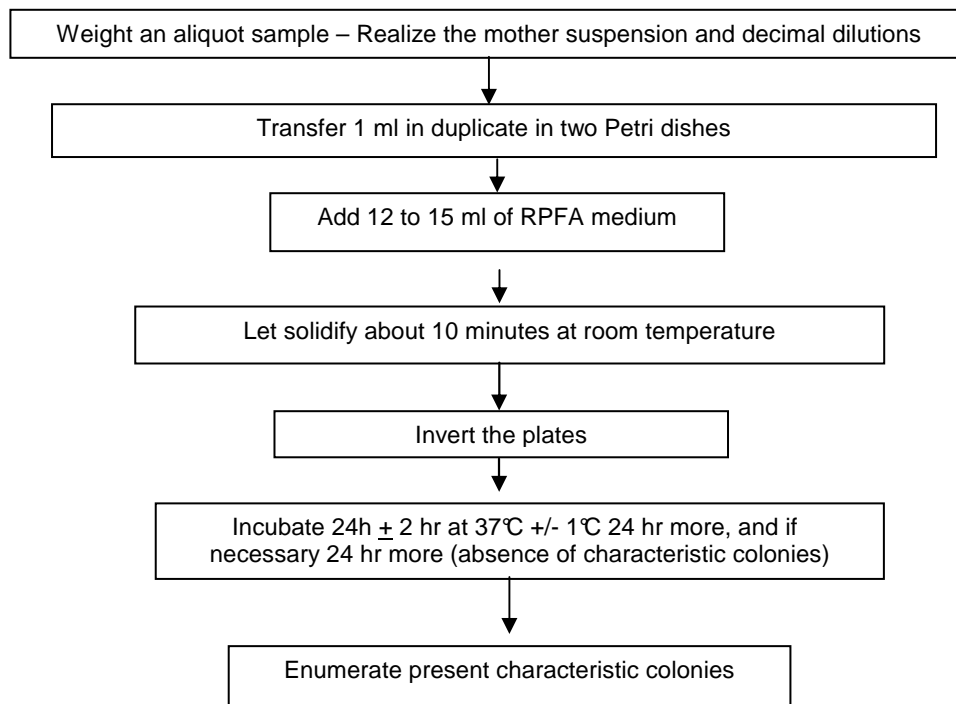
1.4 Reference method

The EN ISO 6888-2:1999 standard and its amendment EN ISO 6888-2/A1:2003, using the rabbit plasma fibrinogen agar medium (RPFA), is the reference method used for the study of AFNOR validation.

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

Analytical diagram is presented on figure 3.

Figure 3: NF EN ISO 6888-2 Standard



1.5 Background of validation

The 3M™ Petrifilm™ Staph Express Count system (STX system) has been validated since April 2003 (certificate n° 3M 01/9-04/03 A).

The reference method for the initial study was the EN ISO 6888-1:1999 standard. An amendment was published in January 2004, including some accuracy data, and the method has not been modified.

Some assays in comparison with the EN ISO 6888-2:1999 standard, with its amendment of 2003, were presented in 2008 and are presented in this report.

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

2 Comparative study

The following criteria were determined :

- linearity
- relative accuracy
- inclusivity and exclusivity
- 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 Nature of the tests

Food products 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 analysed so as to obtain at least 10 usable results in each food category.

The categories and the types of samples studied are the following :

Catégories	Types	Analysed 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 Egg products	With butter cream	8	6
	With custard	6	3
	Egg products	3	1
	TOTAL	17	10
Petfood	Dry food	14	7
	Raw meat	4	4
	Cat/doog 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,
- Uninterpretable results in 23 cases.

2.1.2 Artificial contamination

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 analysed 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).

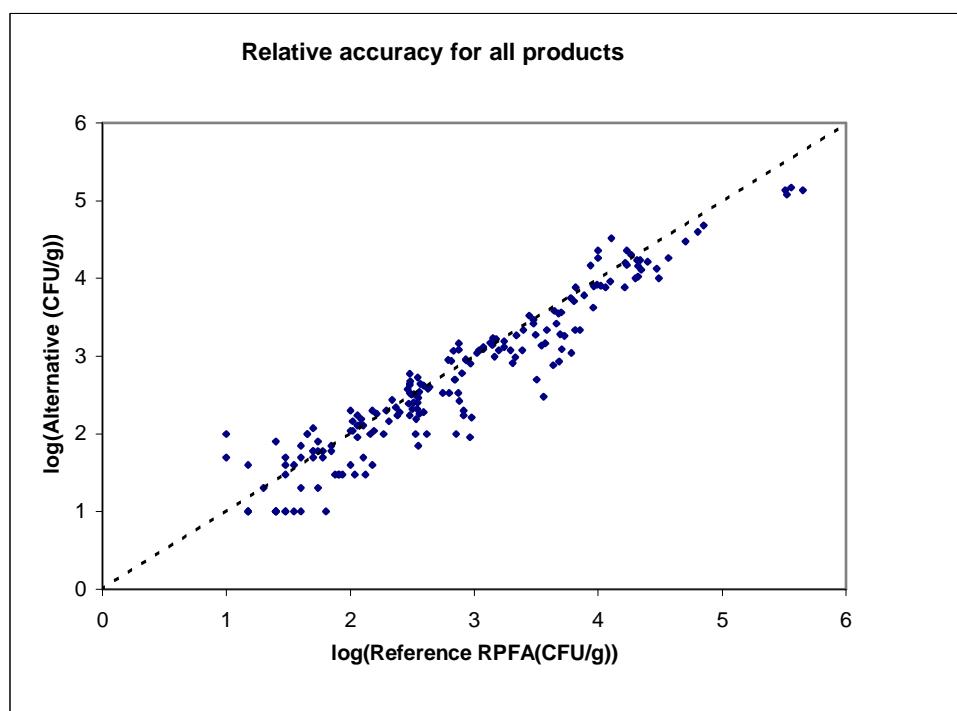
The relative accuracy relationship is evaluated according to the model: $y = bx + a$.

For each of the two methods, robust repeatability standard deviations were calculated (Rob.sr(x) and Rob.sr(y)).

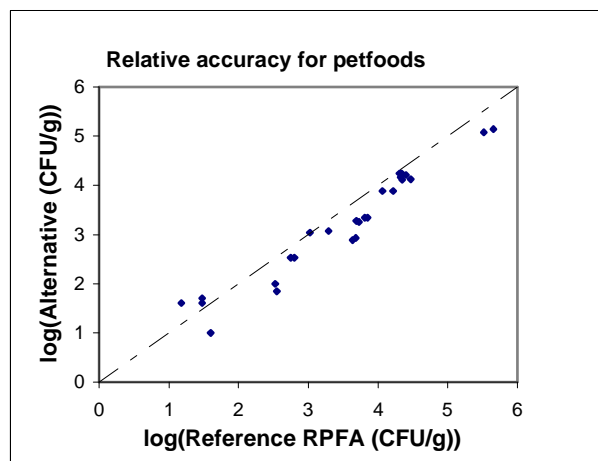
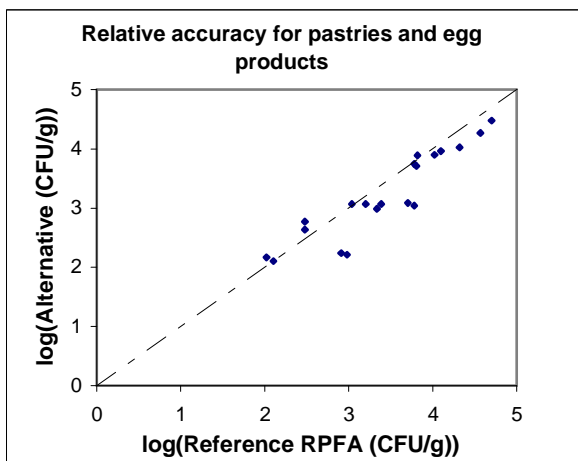
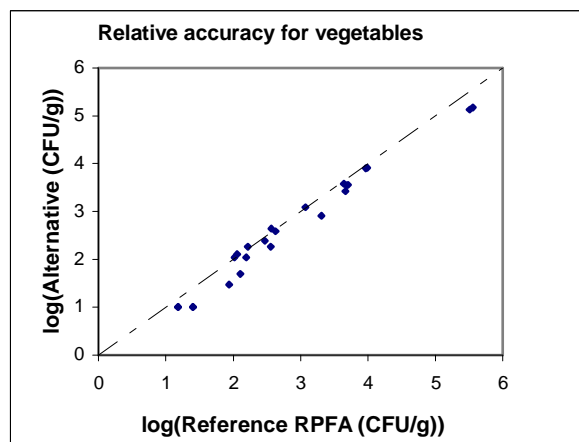
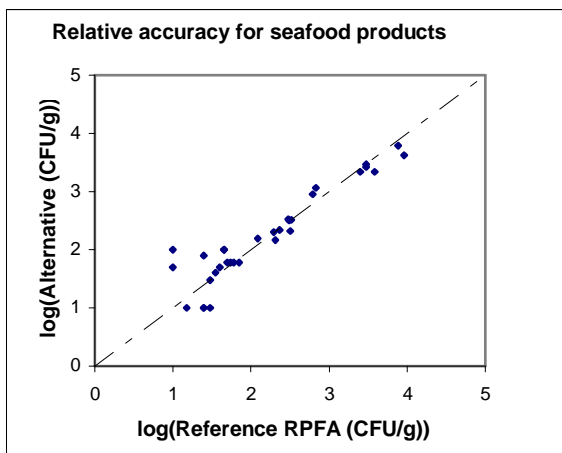
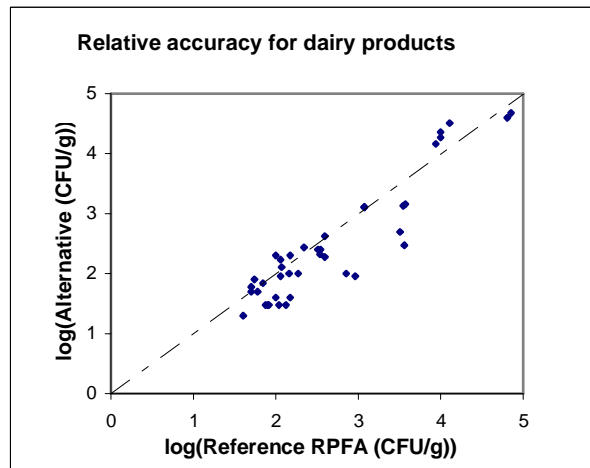
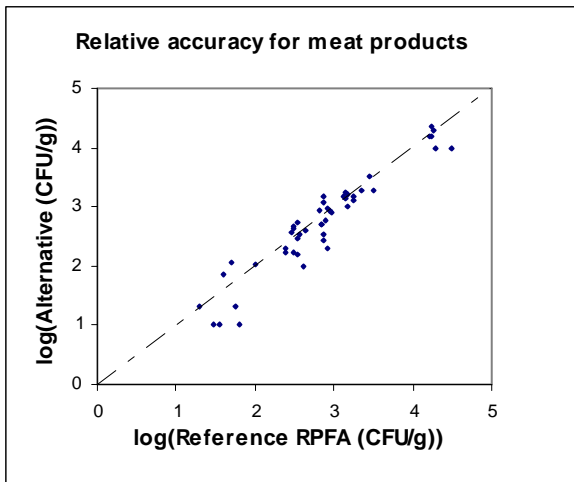
As a function of the ratio of these standard deviations, $Rob.R = Rob.sr(y)/Rob.sr(x)$, the linear regression to be used for the interpretation is defined in the EN ISO 16140 standard.

The following graphs represent the raw values obtained for the samples analysed.

The straight line represented is the first bisector ($y = x$).



The following graphs represent the raw values obtained for the samples analysed in each category.



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 $\alpha = 5\%$:

- **Ordinate at the origin (or intercept) {a = 0}**
The alternative method exhibits a systematic bias compared with the reference method:
 - If the value $t = a / S_a$ with $(q-2)$ degrees of freedom is higher than the T-critical value, obtained in Student's table, or
 - If the probability $p\{a = 0\} < \alpha (=0.05)$, $p\{a = 0\}$ 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 $t = (b-1) / S_b$ with $(q-2)$ degrees of freedom is higher than the T-critical value, obtained in Student's table, or
 - If the probability $p\{b = 1\} < \alpha (=0.05)$, $p\{b = 1\}$ 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
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 all products is the following:

$$\text{Log Alt} = 0.9787 \log \text{Ref} - 0.0827$$

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, is presented in appendix A.

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)
	Réf.	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 à 500	CFU/g
500 à 1000	CFU/g
1000 à 5000	CFU/g
5000 à 10 000	CFU/g
10 000 à 100 000	CFU/g

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

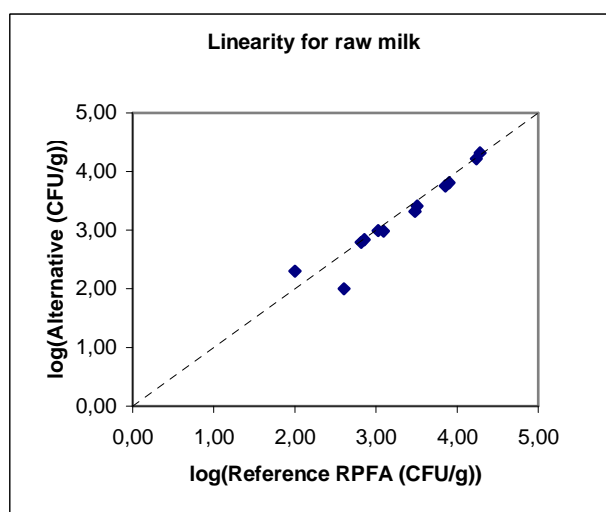
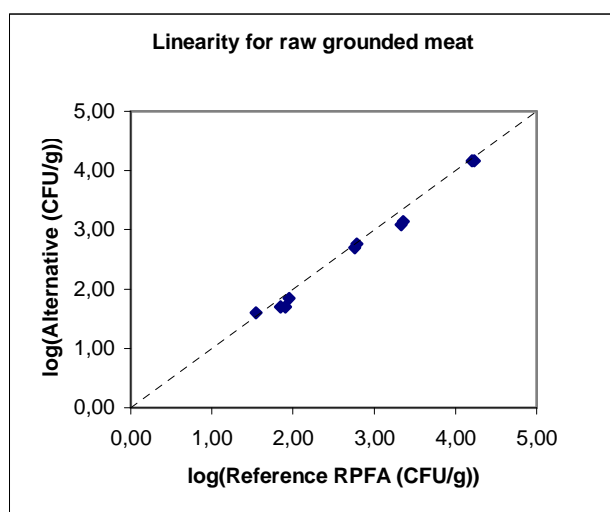
Product	Strain and origin
raw grounded meat	<i>Staphylococcus aureus</i> from grounded raw meat
raw milk	<i>Staphylococcus aureus</i> from yoghourt
raw fish	<i>Staphylococcus aureus</i> from smoked salmon
shredded carrots	<i>Staphylococcus aureus</i> from salad
pet food	<i>Staphylococcus aureus</i> from meat product

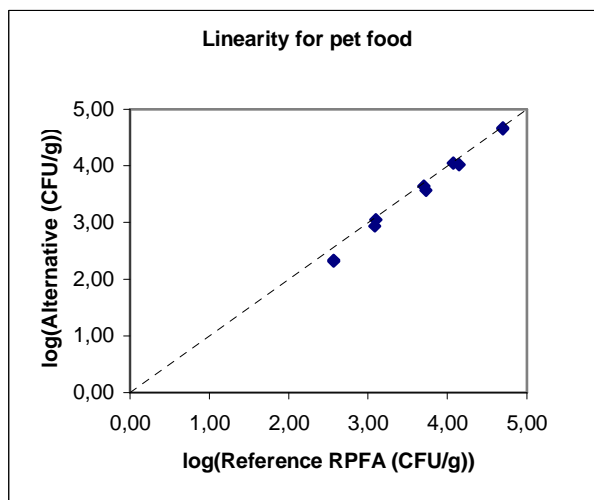
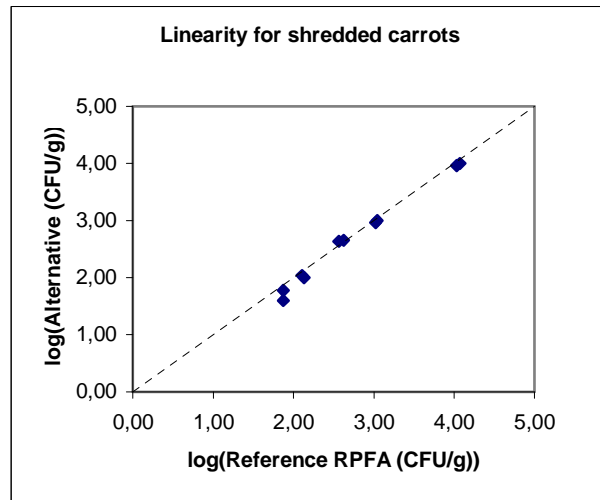
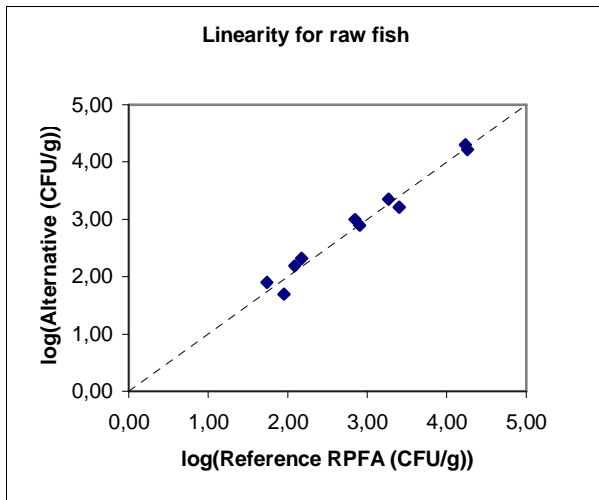
2.2.2 Raw Data

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 :

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

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 (vnum, vden)]
or
- p(F, vnum, vden).< 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 Specificity / selectivity (inclusivity / exclusivity)

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.

2.3.1 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 utilisation conditions, the disk was inserted in all cases. The growth, the coloring and the DNase reaction of colonies were observed.

2.3.2 Results and conclusion

Results are listed in appendix B.

✓ 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 exclusivity 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

3.1 Study organisation

- Number of laboratories

13 laboratories took part in the interlaboratory study.

- Samples

Pasteurised milk has been inoculated by a coagulase-positive *Staphylococcus aureus* strain, isolated from dairy product.

- Number of samples

Height samples were prepared per laboratory, two flasks par inoculation level.

- Analyses

Interlaboratory study laboratories and the expert laboratory have carried out the analyses with the alternative and reference method.

The analyses have been performed two days after sending the samples.

3.2 Verification of experimental parameters

3.2.1 Before spiking

Pasteurized milk was analyzed 5 times according to EN ISO 6888-1 standard, before contaminations, to check initial level of coagulase-positive *Staphylococci* (response <1 CFU /ml according to small number estimation). The matrix didn't contain any coagulase-positive *Staphylococci* (response <1 CFU /ml).

Natural flora of pasteurized milk was 10 CFU/ml.

3.2.2 Contamination levels

The four contamination levels are presented in the following table :

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

3.2.3 Strain stability during transport

In order to evaluate the *Staphylococcus aureus* strain variability during transport, bacterial count of inoculated flasks at level 2, have 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.

3.3 Samples temperature

3.3.1 During transport

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.

3.3.2 On receipt

Measured temperatures on receipt are listed in following table :

Laboratory	Receipt Températures (°C)		Comments
	Measured by the laboratory	Measured by the temperature probe	
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	/

3.3.3 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 of laboratory M had not been exploited.

The results of **12 laboratories** have been included to the statistical interpretations.

3.4 Results

3.4.1 Expert laboratory

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

	EN ISO 6888-1		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.

3.4.2 Collaborative laboratories

Results of the 12 laboratories which realised the analysis are :

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

3.4.3 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 interferent flora with the standard method. No result has been taken into account for this lab.

Therefore, results of 11 laboratories have been statistically exploited.

3.5 Calculations

Statistical interpretations have been calculated according to standard EN ISO 16140, per level of contamination. Results were converted in log for the calculations.

3.5.1 Bias calculation

For each level, difference between duplicate means (d_i) obtained by the alternative method and reference method has been calculated, that allows the determination of ($\text{MED}\{d_i\}$), and the **robust standard deviation** $s\{d_i\}$ ($=k_1 S_n$).

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

$t(d) = \text{MED}\{d_i\} \sqrt{n} / s\{d_i\}$	for n-1 df (degrees of freedom) (n = number of labs) with $\alpha = 5\%$.
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The bias is significant if the $t(d) >$ critical $t_{0,05, df}$ value, i.e; the alternative method lacks accuracy, relative to the reference method for the considered level.

The bias D (alternative – reference) values, the robust standard deviation values ($s\{d_i\}$) and the $t(d)$ values obtained by level are reported in the following table :

	Bias D (log)	s{d_i}	t(d)	$t_{0,05, df}$ value	Conclusion
Level 1	-0.139	0.219	2.103	2.228	{D=0} accepted
Level 2	-0.127	0.039	10.753		{D=0} refused
Level 3	-0.118	0.115	3.399		{D=0} refused

Reminder :

The bias value (alternative – reference) obtained in the comparative study was (– 0.13 log).

Conclusion :

The **{D=0}** hypothesis is statistically accepted for the first level. For the two higher levels, the hypothesis is statistically refused. But, the bias values are similar to those obtained for the first level and obtained in the comparative study.

So, the alternative method accuracy, relative to the reference method is satisfactory.

3.5.2 Repeatability calculation

3.5.2.1 Repeatability limits

For each method and each level, the repeatability limits, r , have been computed : $r = 2.8 S_r$, with S_r : repeatability standard deviation.

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

	r (log CFU/mL) - Reference method	r (log CFU/mL) - Alternative method
Level 1	0.122	0.367
Level 2	0.114	0.125
Level 3	0.147	0.158

3.5.2.2 Interpretation

The repeatability of the alternative and reference method were compared with a F-distribution: $F = (S_{r,alt} / S_{r,réf})^2$ with n and n degrees of freedom. If F (ou $1/F$) > critical $F_{\alpha;n,n}$ value, then the compared methods have different repeatability, for the considered level.

F Values obtained are reported in the following table:

	F (ou 1/F*)	F (0.05 ;n ;n)	n	Conclusion
Level 1	9.11	2.85	11	Different repeatability
Level 2	1.27*			Comparable repeatability
Level 3	1.16			Comparable repeatability

3.5.2.3 Conclusion

The alternative method and reference method repeatability limits are statistically comparable for levels 2 and 3. For the level 1, the repeatability limits are very different.

Reminder :

The repeatability limits obtained during the comparative study were :

- 0.22 log for the reference method,
- 0.23 log for the alternative method.

3.5.3 Reproducibility calculation

3.5.3.1 Reproducibility limits

For each method and each level, the reproducibility limits, R , have been computed : $R = 2.8 S_R$, with S_R : reproducibility standard deviation.

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

	R (log CFU/mL) - Reference method	R (log CFU/mL) - Alternative method
Level 1	0.323	0.554
Level 2	0.240	0.161
Level 3	0.256	0.427

3.5.3.2 Interpretation

The reproducibilities of the alternative and reference method were compared with a F-distribution: $F = (S_{R,alt} / S_{R,réf})^2$ with $n-1$ et $n-1$ degrees of freedom. If F (ou $1/F$) > critical $F_{\alpha;n-1,n-1}$ value, then the compared methods have different repeatability, for the considered level.

F Values obtained are reported in the following table :

	F	F (0.05 ;n-1 ;n-1)	n	Conclusion
Level 1	2.94	2.98	11	Comparable reproducibilities
Level 2	2.23			Comparable reproducibilities
Level 3	2.78			Comparable reproducibilities

3.5.3.3 Conclusion

The alternative method and reference method reproducibility limits are statistically comparable all levels.

4 Practicability

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 6888-1 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 5 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 reponse : presence or not of coagulase-positive <i>Staphylococci</i> in the sample. The analysis of 1 sample according to standard EN ISO 6888-2 gives a result after 24 to 48 hrs 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 tracability	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 Conclusion

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 D1 with regard to D1 or D2 for standard method).

The **comparison** of Petrifilm Staph Express system with EN ISO 6888-2 standard allows to conclude 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.

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

- for all the contamination levels, the alternative method accuracy, relative to the reference method is satisfactory. The bias **{D = 0}** hypothesis is statistically accepted for the first level and the bias obtained for the other levels are similar.

The obtained bias values (alternative– reference) varied by (– 0.12 log) to (– 0.14 log).

The bias value (alternative – reference) obtained in the comparative study was (– 0.13 log).

- the repeatability limits varied by 0.15 to 0.11 log CFU/ml for the reference method and by 0.37 to 0.13 log CFU /ml for the alternative method

And the repeatability limits obtained during the comparative study were 0.22 log for the reference method and 0.23 log for the alternative method.

- the reproducibility limits varied by 0.32 to 0.26 log CFU/ml for the reference method and by 0.55 to 0.16 log CFU /ml for the alternative method. They are statistically comparable for all the levels.

Set of results led to **AFNOR validation** according to ISO 16140, of the 3M Petrifilm Staph Express system (STX) (certificate n°3M 01/9-04/03 B), for the enumeration of coagulase-positive *Staphylococcus* in food products and pet food, **for a 4 years period**.

APPENDICES

APPENDIX A :

RESULTS OF ACCURACY
REGRESSION

	Level	Reference method				Alternative method			
		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	

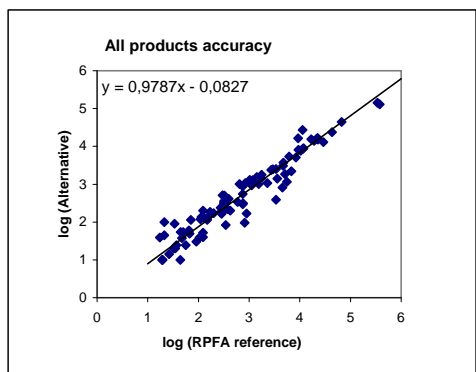
n = 2
q = 95
nq = 190

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

Residual standard deviation from the estimated points by regression

Sy:x = 0,387916365

vi 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	0,06286	
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

Repeatability
= 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 B :

RESULTS OF SPECIFICITY/SELECTIVITY

SPECIFICITY/SELECTIVITY

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