

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 methode EN ISO 6888-1

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 6888-1:2004.

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

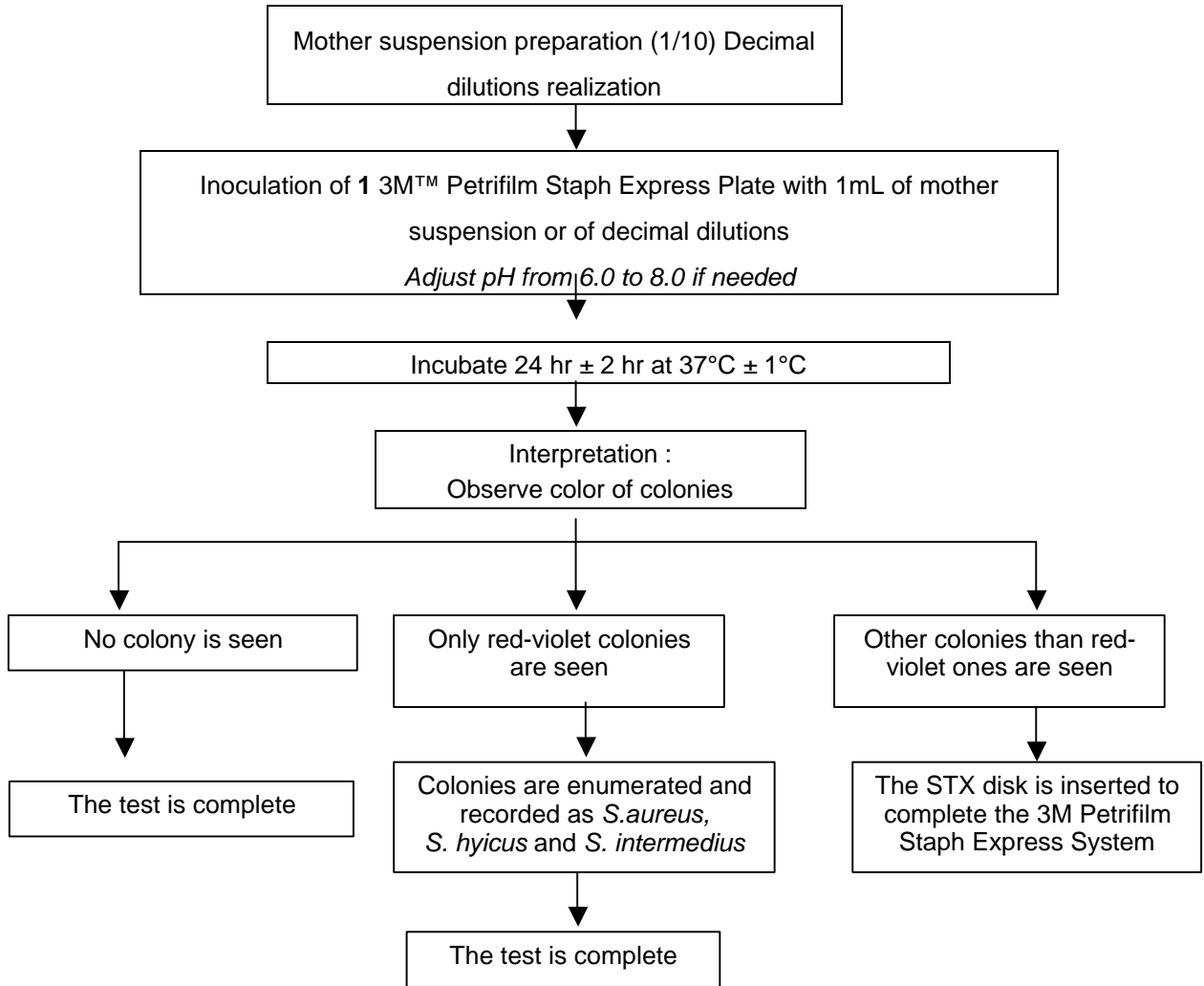
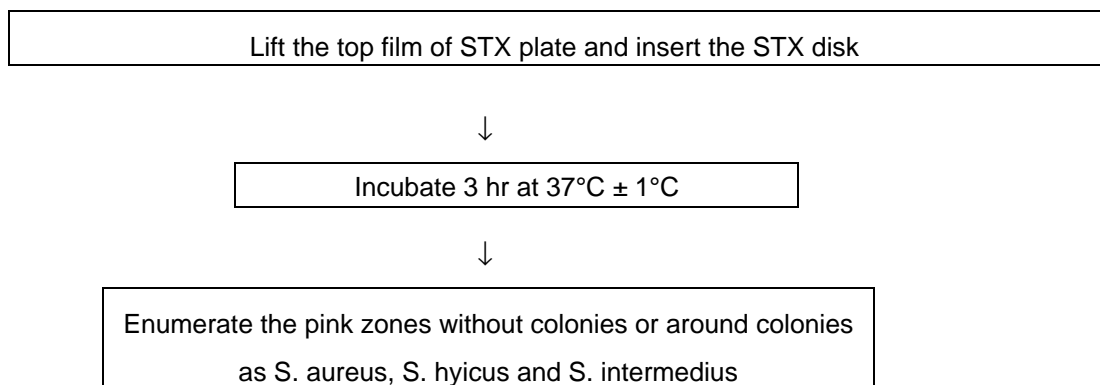


Figure 2 : 3M™ Petrifilm™ Staph Express Disk INSERTION



1.3 Scope

All human food products and pet foods.

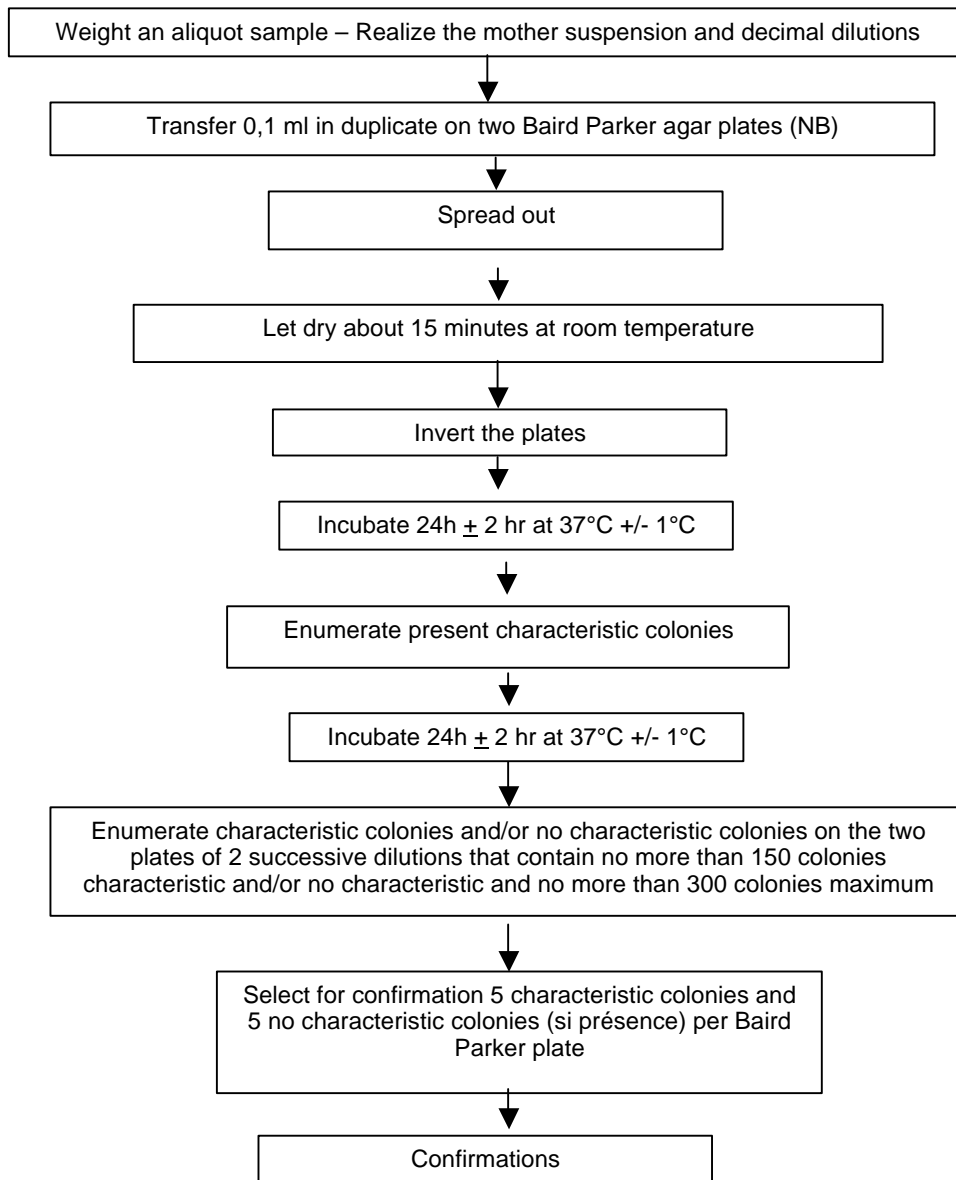
1.4 Reference method

The EN ISO 6888-1 standard, using the Baird-Parker agar medium, is the reference method used for the study of AFNOR validation.

This method is a method with confirmation of the characteristic and not characteristic colonies using coagulase activity test.

Analytical diagrams are presented on figures 3 and 4.

Figure 3 : NF EN ISO 6888-1 Standard
Inoculation protocol

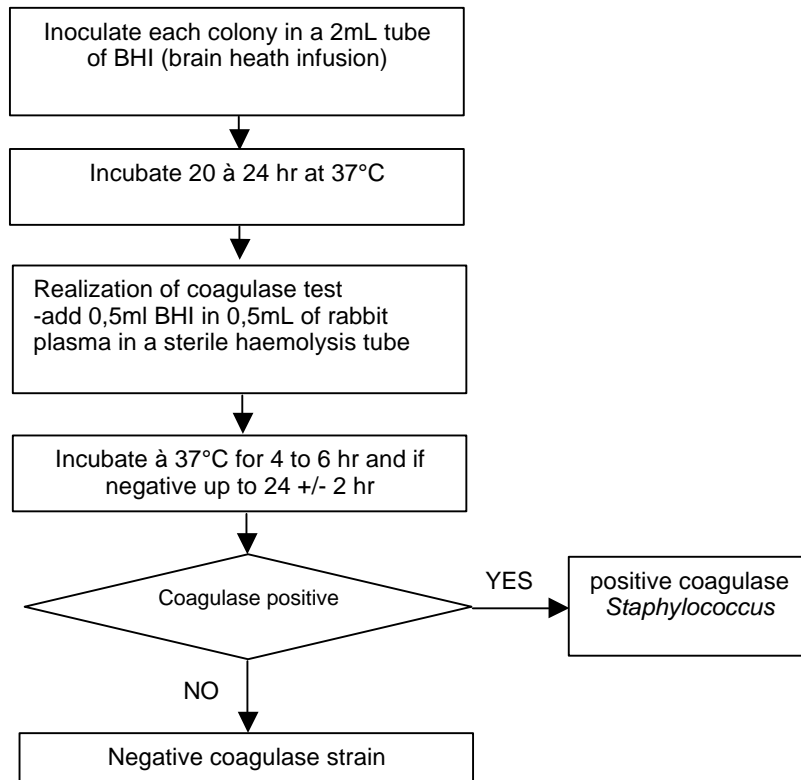


NB : To enumerate low number of *Staphylococcus aureus*, it is possible to inoculate in duplicate 3 BPA plates with 0.3 mL, 0.3 mL and 0.4 mL with the mother suspension or liquid sample (6 dishes).

In this case, enumerate and confirm colonies on all dishes and consider them as two plates for calculations.

During the AFNOR study, we always inoculated 6 BPA dishes (2 x 1 mL) and 2 BPA dishes with 0,1 mL of mother suspension per analyzed sample.

Figure 4 : EN ISO 6888-1
Protocol for confirmation



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).

The reference method 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.

The main elements associated with the Staph Express Count system since 2003 are listed in appendix A. The renewal study was therefore reviewed to conform to the ISO 16140 standard.

Some results of the initial validation study were analysed in comparative study (relative accuracy and linearity). Results of the studies from 2003 were collected in the part showing “relative accuracy”, in the part “linearity” and 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 6888-1, using Baird-Parker agar medium,
- and STX system.

In total, 200 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	27	14
	Prepared & seasoned (raw) meat	12	9
	Charcuteries	15	6
	TOTAL	54	29, with 28 résultats from 2003
Milk products	Raw milk cheeses	30	8
	Raw milk and raw cream	14	9
	Ice cream	4	5
	TOTAL	49	22, with 18 résultats from 2003
Seafood products	Raw fish	10	6
	Shellfish	9	6
	Prepared fish	12	5
	TOTAL	31	17, with 16 résultats from 2003
Vegetables	Raw vegetables	4	2
	Seasoned vegetables	19	11
	Cooked vegetables	4	3
	TOTAL	27	16, with 9 résultats from 2003
Pastries Egg products	With butter cream	8	6
	With custard	6	3
	Egg products	3	1
	TOTAL	17	10, with 10 résultats from 2003
Petfood	Dry food	13	6
	Raw meat	5	5
	Cat/doog food	4	1
	TOTAL	22	12, with 0 résultats from 2003
TOTAL		200	106

The 94 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 12 cases,
- Uninterpretable results in 24 cases.

2.1.2 Artificial contamination

Artificial contamination was achieved on 18 samples by using stressed contaminating suspensions, the stress treatment and efficiency of which have been determined.

The percentage of artificial contamination was globally 17% 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).

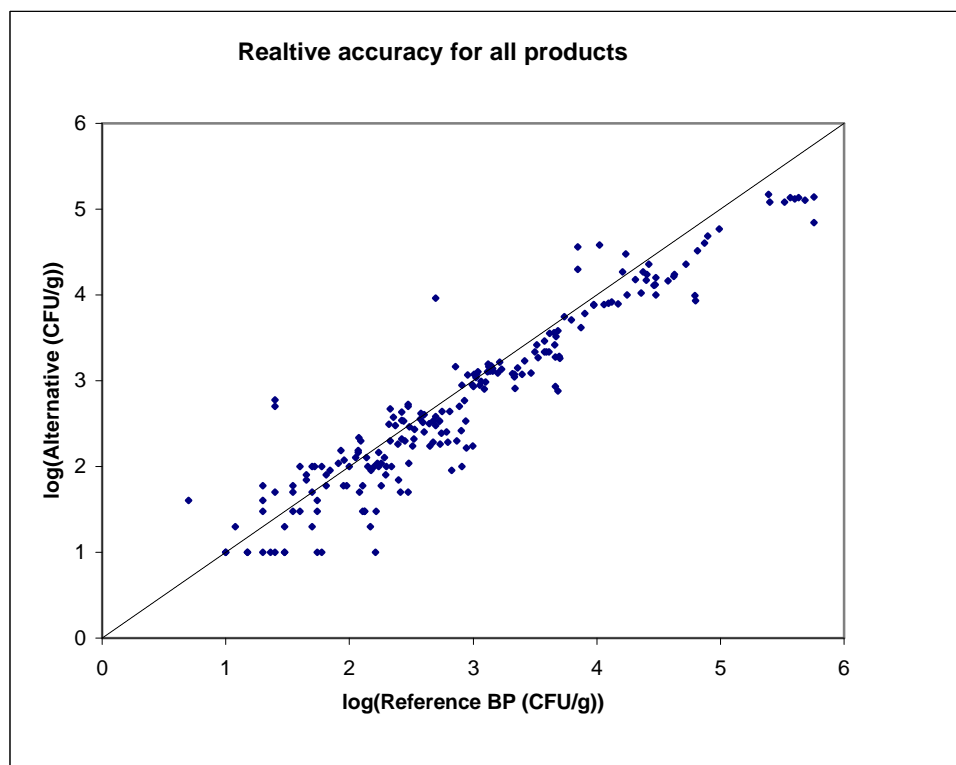
The relative accuracy relationship is evaluated according to the model: $y = bx + a$.

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

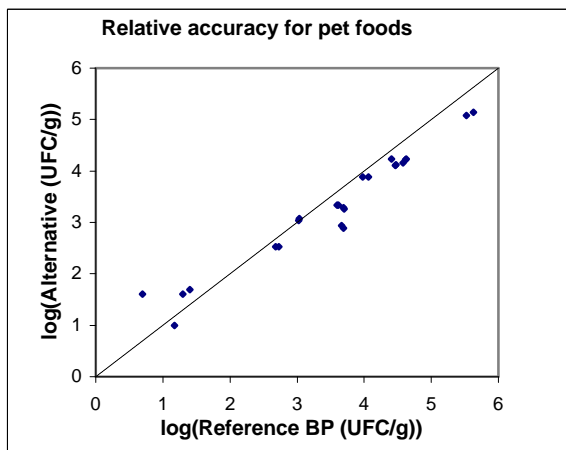
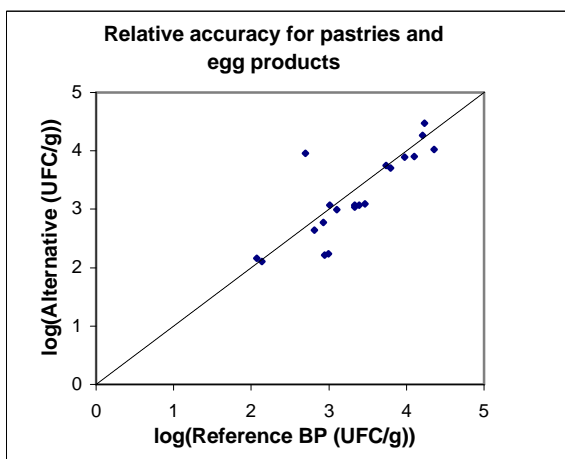
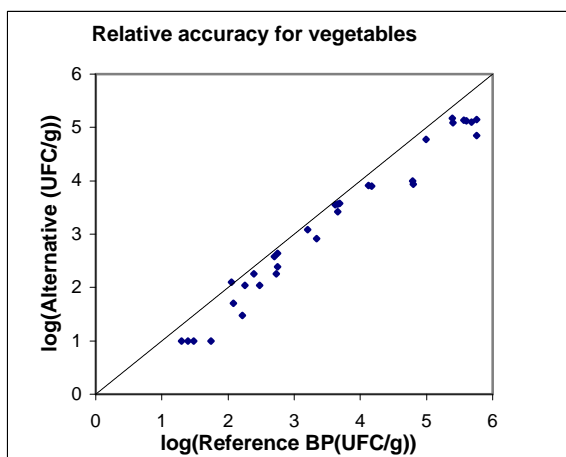
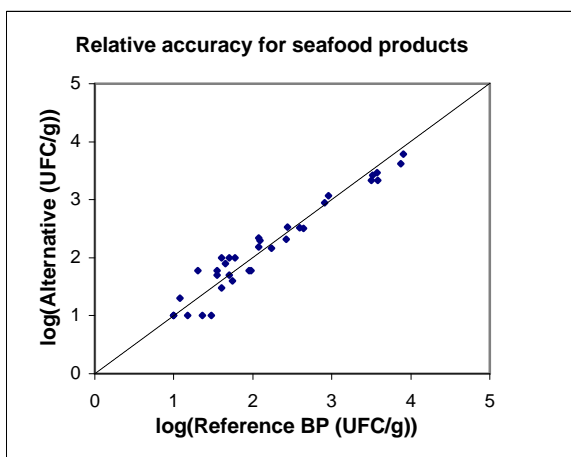
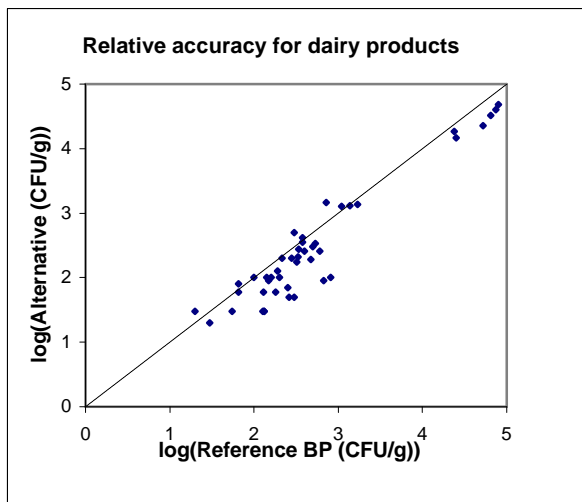
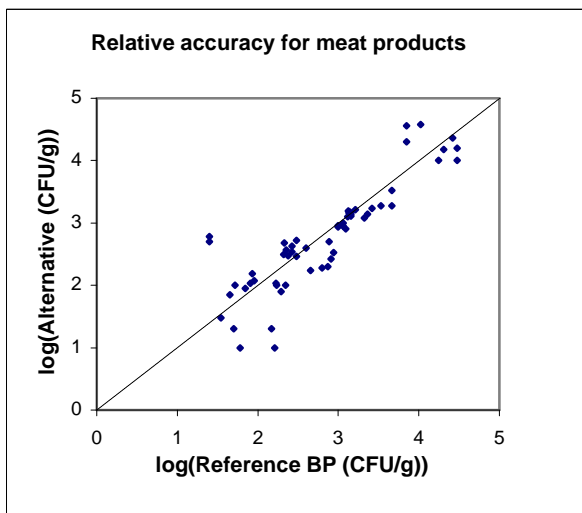
As a function of the ratio of these standard deviations, $Rob.R = Rob.sr(y)/Rob.sr(x)$, the linear regression to be used for the interpretation is defined in the EN ISO 16140 standard.

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	1.091	GMFR	-0.011	0.131	0.896	0.950	1.783	0.077	{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:

$$\text{Log Alt} = 0.9503 \text{ log Ref} - 0.0114$$

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 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)
	Réf.	Alt.	average	median	
Meat products	0.16	0.23	-0.056	-0.077	1.00 - 4.58
Dairy products	0.28	0.30	-0.248	-0.176	1.48 - 4.90
Seafood products	0.17	0.37	+0.002	-0.005	1.00 - 3.90
Vegetables	0.31	0.17	-0.368	-0.363	1.00 - 5.76
Pastries – Egg products	0.24	0.17	-0.109	-0.101	2.10 - 4.48
Pet foods	0.10	0.09	-0.215	-0.277	1.00 - 4.63
All products	0.21	0.23	-0.157	-0.167	1.00 - 5.76

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 0.21 for the alternative method and 0.23 for the reference method.

The bias calculated between the alternative method and the reference method is in the order of $D = -0.16$ 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.

Some results obtained by the two methods in the first validation study in 2003 were used in the statistical analyses, according to EN ISO 16140 standard :

- raw grounded meat,
- raw fish,
- shredded carrots.

For the two other products (raw milk, pet food), analyses were performed in 2007.

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 :

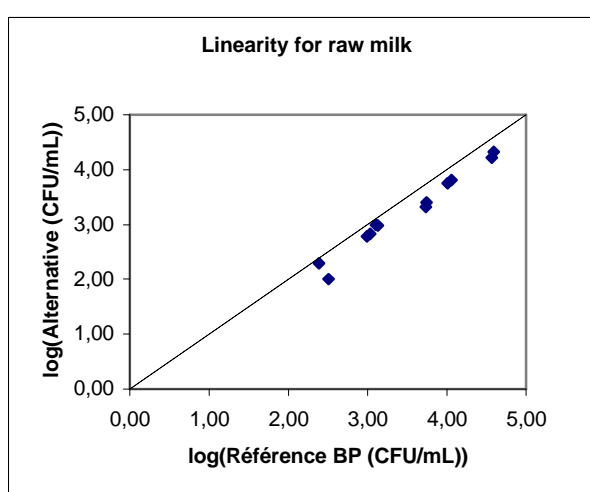
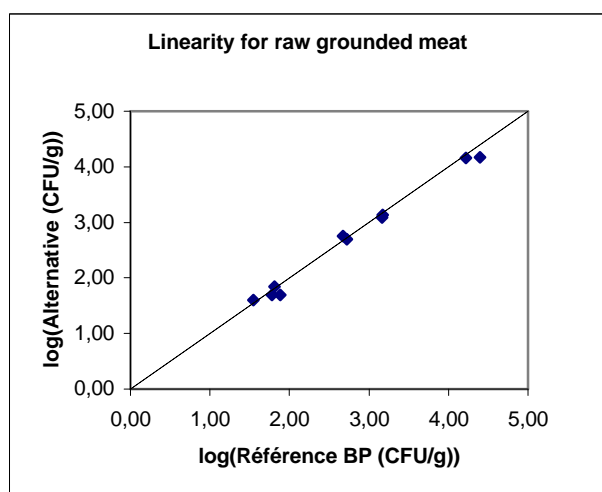
Product	Strain and origin
raw grounded meat	<i>Staphylococcus aureus</i> from grounded raw meat
raw milk	<i>Staphylococcus aureus</i> from yoghourt
raw fish	<i>Staphylococcus aureus</i> from smoked salmon
shredded carrots	<i>Staphylococcus aureus</i> from vegetables salad
pet food	<i>Staphylococcus aureus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

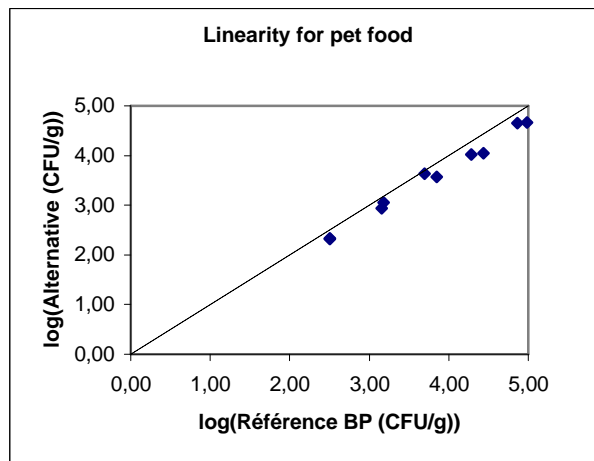
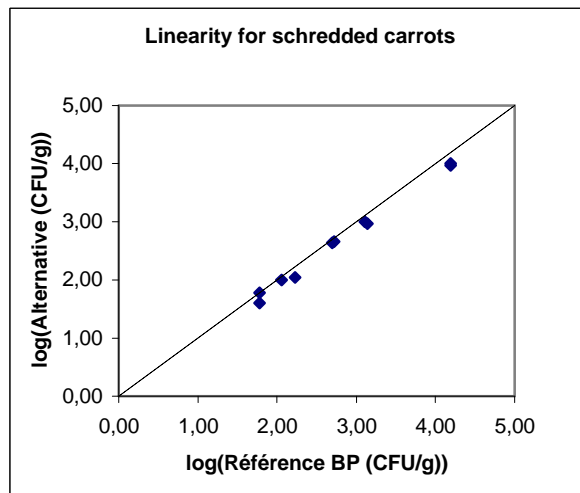
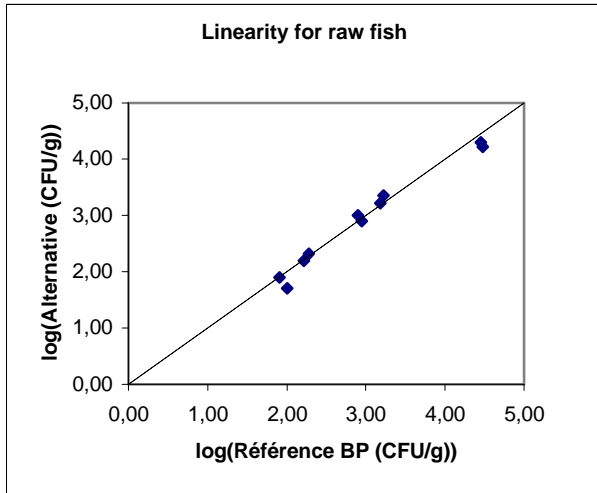
2.2.2 Raw Data

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	0.855	GMFR	5.41	3.651	10 %
raw milk	1.870	GMFR	5.19	5.838	3 %
raw fish	2.933	OLS1	5.41	3.415	11 %
shredded carrots	1.684	GMFR	5.41	6.188	4 %
pet food	0.211	OLS2	5.41	68.469	0 %

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

raw grounded meat	Log Alt = 0.9621 log Ref + 0.0526	R ² = 0.997
raw milk	Log Alt = 0.9719 log Ref - 0.1634	R ² = 0.985
raw fish	Log Alt = 0.9624 log Ref + 0.0626	R ² = 0.978
shredded carrots	Log Alt = 0.9548 log Ref + 0.0041	R ² = 0.998
pet food	Log Ref = 1.0569 log Alt - 0.4343	R ² = 0.993

2.2.4 Conclusion

The statistical tests conclude that the relationship between the alternative method and the reference method is linear for "raw grounded meat" and "raw fish".

For « raw milk » and « shredded carrots », the p-values are 3% and 4% : the non-linearity test is significant.

For « 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 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 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 methods.

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 6888-1 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	85	75	40	40
Level 2	620	480	370	600
Level 3	6700	7000	6100	6200

Results according to standard EN ISO 6888-1 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 6888-1		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	<100	<100	<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	<10	<10	<10	<10

Level 1 (results in CFU/ml)

Lab	EN ISO 6888-1		STX system	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	110	50	80	100
B	65	25	40	90
C	85	35	40	90
D	75	35	70	90
E	40	10	40	40
F	50	100	70	60
G	30	70	30	40
H	60	35	80	60
I	45	100	80	170
J	<10	60	110	70
K	70	100	50	50
L	65	60	90	60
M	<10	<10	<10	60

Level 2 (results in CFU/ml)

Lab	EN ISO 6888-1		STX system	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	270	190	590	720
B	370	400	500	620
C	880	970	580	710
D	990	930	780	830
E	700	790	560	680
F	1100	1100	670	710
G	900	530	630	670
H	800	800	920	860
I	550	750	600	860
J	Pb of protocol	210	520	540
K	670	800	680	620
L	840	920	680	740
M	Pb of protocol	Pb of protocol	740	600

Level 3 (results in CFU/ml)

Lab	EN ISO 6888-1		STX system	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
A	2900	3400	7900	4600
B	6000	3700	5500	4800
C	13000	11000	9700	7600
D	7600	12000	8500	10000
E	5800	9900	5500	5800
F	9600	9200	4100	6300
G	9600	6800	6500	7500
H	13000	5400	8500	8900
I	10000	10000	11000	10000
J	1500	3200	5200	5100
K	10000	12000	6300	7100
L	10000	11000	8400	7500
M	Pb of protocol	Pb of protocol	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 J** obtained a “<10 CFU/ml” count for one duplicate of level 1 and encountered problems 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 ($=MED\{d_i\}$), 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 :

$t(d) = MED\{d_i\} \sqrt{n} / s\{d_i\}$	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	0.091	0.171	1.774	2.228	{D=0} accepted
Level 2	-0.050	0.144	1.157		{D=0} accepted
Level 3	-0.064	0.161	1.312		{D=0} accepted

Reminder :

The bias value (alternative – reference) obtained in the comparative study was (– 0.17 log).

Conclusion :

The **{D = 0}** hypothesis is statistically accepted for all the levels.

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	0.972	0.367
Level 2	0.125	0.125
Level 3	0.265	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	7.18*	2.85	11	Different repeatability
Level 2	1.00			Comparable repeatability
Level 3	2.80*			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, due to the high value obtained for the reference method (some labs encountered difficulties to perform the reference method with 1 mL on 3 plates of Baird Parker).

Reminder :

The repeatability limits obtained during the comparative study were :

- 0.21 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	0.825 → 0.972 (r)	0.482
Level 2	0.398	0.161
Level 3	0.372	0.393

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	4.07	2.98	11	Different reproducibilities
Level 2	6.10			Different reproducibilities
Level 3	1.11			Comparable reproducibilities

3.5.3.3 Conclusion

The alternative method and reference method reproducibility limits are statistically comparable for the level 3. For the levels 1 and 2, the reproducibility limits are different, due to the higher values obtained for the reference method.

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 8 minutes maximum per positive sample in comparison with standard method which needs tests of confirmation (coagulase activity of 20 colonies at the most). 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 6888-1 gives a result : - after 48 hrs (D2) for a sample which doesn't give any characteristic or not characteristic colony on Baird-Parker agar medium - after 96 hrs (D4) for a sample with confirmations
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 D4 for standard method).

The **comparison** of Petrifilm Staph Express system with EN ISO 6888-1 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 all the levels.
The obtained bias values (alternative– reference) varied by (+ 0.09 log) to (– 0.05 log).
The bias value (alternative – référence) obtained in the comparative study was (– 0.17 log).
- the repeatability limits varied by 0.97 to 0.13 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 0.21 log for the reference method and 0.23 log for the alternative method.
- the reproducibility limits varied by 0.97 to 0.27 log CFU/ml for the reference method and by 0.48 to 0.16 log CFU /ml for the alternative method. They are statistically comparable for the level 3 and higher for the reference method in the levels 1 and 2.

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

APPENDICES

APPENDIX A :

MAIN RESULTS OF THE FIRST VALIDATION STUDY

- 1st Validation date

The method has been validated for human foods since April 2003, with the certificate number 3M 01/9-04/03.

- Reference method used for the validation

Method EN ISO 6888-1 :1999 and EN ISO 6888-1/A1 :2004, using the Baird-Parker agar medium

This method is a method with confirmation of the characteristic and not characteristic colonies using coagulase activity test.

- Main results obtained in the first validation study

Specificity

The aim of this study is to check that all *S. aureus* 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.

22 strains of *S. aureus* and 11 other strains have been tested.

✓ 3M™ Petrifilm™ Staph Express enumeration system permitted to detect all *Staphylococcus aureus* inoculated strains. All colonies had a typical aspect after incubation and after revelation with STX disk.

✓ Among the other *Staphylococci*, only *S. hyicus* and *S. intermedius*, which are positive coagulase strains, presented typical aspect as *Staphylococcus aureus*.

✓ Strains of other genus didn't give any culture or typical colonies (no red-violet color, no pink zone after disk insertion).

Linearity

Four food matrices (raw milk, minced meat, raw fish filet, and shredded carrots), artificially contaminated with 4 *Staphylococcus aureus* strains at 5 levels (from 50/g and 10⁵/g), were analyzed in duplicate according to reference method ISO 6888-1 and STX method.

Les paramètres des droites de régression obtenues étaient :

		Raw milk	Minced meat	Raw fish filet	Carottes râpées
Sensibility (slope)	b1 =	0.95	1.00	1.01	0.97
Sensibility standard deviation	s(b1) =	0.03	0.04	0.04	0.02
Analytical blank (origin ordinate)	b0 =	0.14	-0.05	-0.00	0.08
Analytical blank standard deviation	s(b0) =	0.09	0.13	0.12	0.07
Experimental variance	s ² (e) =	0.02	0.03	0.02	0.01
Experimental standard deviation	s (e) =	0.13	0.18	0.15	0.09
Correlation factor	R =	0.99	0.98	0.99	0.99

According to statistical results obtained with norm NF V 03 110, Petrifilm Staph Express enumeration system give **linear results**, whatever the food matrix.

Regression curves allow to check that points are correctly put together along ($y = x$).

Accuracy

205 products were analyzed to obtain 126 exploited results.

Les produits étaient répartis dans les catégories suivantes :

Categories of products	Number of analyzed products	Number of exploited products
Meat products	62	35
Dairy products	50	31
Seafood products	49	35
Vegetables and other food products	44	25

All results, converted into log(N), have been analyzed according to NF V 03 110 standard.

Among these products, some have been artificially contaminated (42 %, that is to say 53/126) by :

- contact with naturally contaminated products,
- inoculation with a stressed *Staphylococcus aureus* strain.

The main results were :

	Alternative method	Reference method
Means	X = 2.71	Z = 2.84
Repeatability standard deviation	$s^r(x) = 0.13$	$s^r(z) = 0.13$
Repeatability variance	$s^{2r}(x) = 0.02$	$s^{2r}(z) = 0.02$
Repeatability	r = 0.36	r = 0.36

Verification tests for the accuracy and the repeatability

	Obtained values	Critical value	Conclusion
Means difference	X-Z = -0.13		
Means difference standard deviation	s(d) = 0.22		
Ratio	w = 0.59	3.00	Acceptable
Ratio of repeatability variance	q = 0.98	1.00	Acceptable

The alternative method showed an accuracy as good as the reference method.

Fidelity

Values of repeatability and of reproducibility were obtained after an interlaboratory study with 14 laboratories and varied by:

- 0.14 to 0.33 log CFU/ml for the repeatability according the levels of contamination.
- 0.20 to 0.37 log CFU/ml for the reproducibility according the levels of contamination.

- Bibliography

- Validation AOAC OMA 2003.07 (Journal of AOAC)
3M Petrifilm Staph Express Plate Method for the Enumeration of *Staphylococcus aureus* in selected types of processed and prepared foods
- Validation AOAC OMA 2003.08 (Journal of AOAC)
3M Petrifilm Staph Express Plate Method for the Enumeration of *Staphylococcus aureus* in selected dairy foods
- Validation AOAC OMA 2003.11 (Journal of AOAC)
3M Petrifilm Staph Express Plate Method for the Enumeration of *Staphylococcus aureus* in selected Meat, Seafood and Poultry

- Technical requirements for AFNOR validation

The technical requirement was this of 2002-2003 and asked for the NF V03-110. Now, it is replaced by EN ISO 16140 :2003.

APPENDIX B :

RESULTS OF ACCURACY
REGRESSION

	Level	Reference method				Alternative method			
		Rep.1	Rep.2	Mxi	sxi	Rep.1	Rep.2	Myi	syi
Meat products	1	2.21	2.17	2.18979	0,03064	1,00	1,30	1.15051	0,21286
	2	2.65	2.79	2.72363	0,10083	2,24	2,28	2.25909	0,03073
	3	1.40	1.40	1.39794	0,00000	2,70	2,78	2.73856	0,05599
	4	2.36	2.42	2.38878	0,04558	2,57	2,54	2.55489	0,02333
	5	2.23	1.96	2.09116	0,19038	2,04	2,07	2.05517	0,02458
	6	2.34	2.60	2.47224	0,18359	2,00	2,60	2.30103	0,42572
	7	3.67	3.67	3.66703	0,00180	3,28	3,52	3.39759	0,17101
	8	3.36	3.32	3.33974	0,02744	3,15	3,08	3.11265	0,04734
	9	3.42	3.53	3.47278	0,07641	3,23	3,27	3.25041	0,02822
	10	3.13	3.09	3.10971	0,02710	3,16	2,90	3.03291	0,18359
	11	3.06	3.06	3.06000	0,00438	3,00	2,95	2.97071	0,03582
	12	3.12	3.16	3.13640	0,02834	3,10	3,14	3.12259	0,02525
	13	1.85	1.91	1.87666	0,04464	1,95	2,04	1.99602	0,05908
	14	2.94	2.91	2.92321	0,02498	2,53	2,42	2.47391	0,07481
	15	4.24	3.85	4.04407	0,28139	4,00	4,30	4.15051	0,21286
	16	4.48	4.42	4.45018	0,03810	4,00	4,36	4.18086	0,25578
	17	4.48	4.31	4.39444	0,11693	4,20	4,18	4.19011	0,01982
	18	1.78	1.54	1.66111	0,16552	1,00	1,48	1.23856	0,33738
	19	4.02	3.85	3.93314	0,12452	4,58	4,56	4.56804	0,01660
	20	3.12	3.16	3.13942	0,02328	3,19	3,11	3.15277	0,05491
	21	2.33	2.48	2.40340	0,10426	2,67	2,72	2.69832	0,03353
	22	2.48	2.42	2.45233	0,04430	2,46	2,63	2.54723	0,11805
	23	2.89	2.87	2.87890	0,01291	2,70	2,30	2.50000	0,28139
	24	3.21	3.15	3.18010	0,04606	3,22	3,17	3.19354	0,03217
	25	1.65	1.70	1.67609	0,03236	1,85	1,30	1.57306	0,38471
	26	2.99	3.00	2.99702	0,00422	2,96	2,94	2.94747	0,01575
	27	2.37	2.32	2.34696	0,03765	2,48	2,49	2.48360	0,00917
	28	1.93	1.72	1.82611	0,15124	2,19	2,00	2.09453	0,13368
	29	2.29	2.24	2.26420	0,03796	1,90	2,00	1.95154	0,06853
Dairy products	1	2.78	2.67	2.72965	0,07783	2,41	2,28	2.34330	0,08835
	2	2.18	2.12	2.14803	0,03968	1,95	1,48	1.71568	0,33738
	3	1.81	1.74	1.77664	0,05130	1,90	1,48	1.69011	0,30121
	4	2.10	2.12	2.11236	0,01078	1,48	1,48	1.47712	0,00000
	5	3.04	3.14	3.08769	0,07057	3,10	3,11	3.10934	0,00651
	6	2.00	2.15	2.07447	0,10532	2,00	2,00	2.00000	0,00000
	7	2.30	2.28	2.29179	0,01307	2,00	2,10	2.05237	0,07406
	8	2.60	2.58	2.58936	0,01796	2,41	2,62	2.51357	0,15245
	9	2.51	2.20	2.35524	0,21722	2,24	2,00	2.11868	0,16784
	10	2.33	2.45	2.38982	0,08506	2,30	2,30	2.30103	0,00000
	11	2.70	2.48	2.58805	0,15687	2,48	2,70	2.58805	0,15687
	12	2.40	2.48	2.43753	0,05599	1,85	1,70	1.77203	0,10333
	13	2.41	2.26	2.33551	0,11023	1,70	1,78	1.73856	0,05599
	14	2.91	2.83	2.86792	0,05668	2,00	1,95	1.97712	0,03236
	15	2.73	2.57	2.65090	0,10797	2,53	2,55	2.53824	0,01617
	16	1.81	2.11	1.96103	0,20947	1,78	1,78	1.77815	0,00000
	17	4.40	4.37	4.38576	0,01722	4,17	4,27	4.21711	0,06928
	18	2.53	2.52	2.52385	0,00418	2,44	2,32	2.37803	0,08160
	19	4.87	4.90	4.88395	0,02005	4,60	4,68	4.64247	0,05715
	20	4.72	4.81	4.76747	0,06426	4,36	4,51	4.43573	0,11198
	21	3.23	2.86	3.04471	0,26267	3,13	3,16	3.14871	0,01982
	22	1.48	1.30	1.38908	0,12452	1,30	1,48	1.38908	0,12452
Seafood products	52	1.08	1.36	1.22045	0,19979	1,30	1,00	1.15051	0,21286
	53	1.48	1.00	1.23856	0,33738	1,00	1,00	1.00000	0,00000
	54	2.91	2.96	2.93221	0,03425	2,95	3,07	3.00783	0,08201
	55	1.70	1.60	1.65051	0,06853	2,00	2,00	2.00000	0,00000
	56	3.90	3.87	3.88643	0,02356	3,78	3,62	3.70302	0,11548
	57	3.58	3.58	3.57796	0,00111	3,46	3,34	3.40129	0,08835
	58	1.54	1.60	1.57306	0,04101	1,70	1,48	1.58805	0,15687
	59	2.24	2.09	2.16506	0,10225	2,16	2,30	2.23188	0,09779
	60	1.98	1.95	1.96598	0,01660	1,78	1,78	1.77815	0,00000
	61	1.65	1.78	1.71568	0,08835	1,90	2,00	1.95154	0,06853
	62	1.00	1.30	1.15051	0,21286	1,00	1,78	1.38908	0,55024
	63	1.70	1.54	1.62152	0,10953	1,70	1,78	1.73856	0,05599
	64	3.52	3.50	3.50750	0,01388	3,42	3,34	3.37991	0,05811
	65	2.59	2.64	2.61821	0,03696	2,51	2,50	2.50879	0,00865
	66	2.44	2.42	2.42837	0,01041	2,53	2,32	2.42357	0,14600
	67	2.08	2.07	2.07414	0,00224	2,34	2,19	2.26394	0,10590
	68	1.74	1.18	1.45823	0,39900	1,60	1,00	1.30103	0,42572
	Vegetables	69	2.21	2.08	2.14653	0,09525	1,48	1,70	1.58805
70		3.20	3.34	3.26870	0,09659	3,09	2,91	3.00090	0,12452
71		2.73	2.74	2.73853	0,00765	2,26	2,39	2.32480	0,09216
72		2.26	2.39	2.32480	0,09216	2,04	2,26	2.14871	0,15687
73		2.05	2.48	2.26402	0,30477	2,10	2,04	2.07126	0,04734
74		1.74	1.30	1.52070	0,31066	1,00	1,00	1.00000	0,00000
75		2.75	2.70	2.72498	0,03679	2,64	2,58	2.61085	0,04101
76		3.66	3.62	3.63894	0,02820	3,42	3,55	3.48534	0,09098
77		3.66	3.69	3.67101	0,02084	3,56	3,58	3.57126	0,01498
78		1.48	1.40	1.43753	0,05599	1,00	1,00	1.00000	0,00000
79		4.17	4.12	4.14605	0,03688	3,90	3,92	3.90789	0,01380
80		5.99	5.57	5.47802	0,12452	5,17	5,13	5.15129	0,02758
81		4.80	4.79	4.79745	0,00445	3,93	3,99	3.96188	0,04264
82		5.68	5.76	5.72042	0,05308	5,10	5,14	5.12402	0,02727
83		5.40	5.60	5.50000	0,14433	5,08	5,12	5.10122	0,02653
84		4.99	5.76	5.37398	0,54301	4,77	4,85	4.80831	0,05203
Pastries	85	2.07	2.14	2.10482	0,05038	2,16	2,10	2.13373	0,04101
	86	3.97	4.36	4.16505	0,27082	3,89	4,02	3.95555	0,09549
	87	4.24	4.21	4.22205	0,01841	4,48	4,27	4.37161	0,14921
	88	4.10	2.70	3.39794	0,98849	3,90	3,96	3.93301	0,04231
	89	3.79	3.74	3.76439	0,03959	3,71	3,75	3.72788	0,02872
	90	3.00	2.95	2.97182	0,03424	2,24	2,21	2.22562	0,01660
	91	3.47	3.34	3.40195	0,09314	3,09	3,04	3.06517	0,03362
	92	3.01	3.10	3.05316	0,06411	3,07	2,99	3.03027	0,05979
	93	2.81	2.93	2.87117	0,08238	2,64	2,77	2.70568	0,09311
	94	3.39	3.33	3.36314	0,04472	3,07	3,07	3.07255	0,00000
	Pet foods	95	4.63	4.62	4.62604	0,00660	4,24	4,21	4.22562
96		4.58	4.41	4.49121	0,12084	4,16	4,24	4.20004	0,05277
97		1.30	1.18	1.23856	0,08835	1,60	1,00	1.30103	0,42572
98		3.69	3.67	3.67657	0,01470	2,88	2,93	2.90731	0,03454
99		1.40	0.70	1.04846	0,49425	1,70	1,60	1.65051	0,06853
100		2.73	2.68	2.70226	0,03323	2,53	2,53	2.52681	0,00000
101		3.59	3.61	3.60195	0,01396	3,34	3,34	3.33882	0,00000
102		3.70	3.70	3.69895	0,00558	3,26	3,28	3.27023	0,01498
103		5.52	5.63	5.57580	0,07764	5,08	5,14	5.11002	0,03898
104		4.47	4.46	4.46712	0,00476	4,12	4,11	4.11693	0,00853
105		3.02	3.03	3.02678	0,00525	3,04	3,07	3.05517	0,02458
106		4.06	3.97	4.01712	0,06162	3,89	3,88	3.88546	0,00363

Median x	2.73409	0.05084
Mean x	2.92368	
Srx	0.16411	
Rob Swx	0.07538	
R	0.86278	
Rob.R	1.09066	

Median y	2.55106	0.05545
Mean y	2.76691	
Sry	0.14159	
Rob Swy	0.08221	

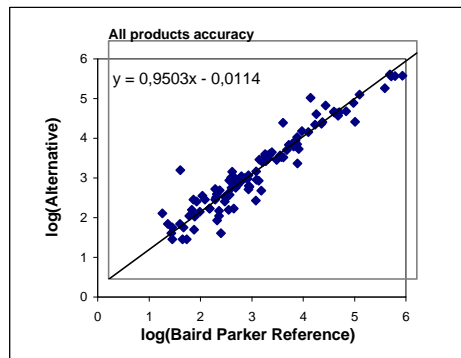
n = 2
q = 106
nq = 212

$0.5 < R < 2$	GMFR
$R > 2$	OLS
$R < 0.5$	OLS chgt

GMFR regression

Global standard deviations

Vxi	Vyi	
1,07811	5,27079	
0,09020	0,51670	
4,65574	0,00474	
0,57431	0,09045	
1,42242	1,01376	
0,44129	0,61533	
1,10514	0,82476	
0,34697	0,24132	
0,60887	0,46833	
0,06995	0,17521	
0,03719	0,08435	
0,09131	0,25366	
2,19446	1,19205	
0,00062	0,17730	
2,58973	3,87403	
4,66190	4,06395	
4,33995	4,05135	
3,21555	4,78554	
2,05355	6,48842	
0,09364	0,30079	
0,55225	0,01053	
0,44630	0,11045	
0,00418	0,22166	
0,13363	0,36506	
3,11398	2,99855	
0,01078	0,06545	
0,66663	0,16061	
2,43216	0,92207	
0,87125	1,33434	
0,08135	0,36671	
1,20482	2,32399	
2,63403	2,40975	
1,31660	3,32712	
0,05878	0,23456	
1,45340	1,17631	
0,79874	1,02663	
0,22386	0,15161	
0,69342	0,86858	
0,57724	0,43409	
0,24990	0,08859	
0,47581	1,99024	
0,70403	2,11815	
0,00943	1,24858	
0,16047	0,10484	
1,89726	1,95529	
4,27567	4,21097	
0,31973	0,30911	
7,68571	7,03872	
6,80331	5,58244	
0,09830	0,29194	
4,72550	3,81237	
5,84185	5,27079	
5,79305	6,24395	
0,00132	0,12280	
3,24658	1,17631	
1,85433	1,76595	
0,85618	0,81267	
3,64999	2,80406	
1,16146	0,58208	
1,83463	1,95529	
2,92631	1,33434	
6,33351	4,09962	
3,40323	2,11815	
0,68189	0,75492	
0,18798	0,13333	
0,49077	0,25708	
1,44344	0,51718	
4,45429	4,47886	
1,21698	2,80406	
0,24742	0,12500	
0,06862	0,39941	
0,72579	0,78895	
0,96318	0,97010	
4,03321	6,24395	
0,08031	0,05039	
1,02399	1,04065	
1,11744	1,29418	
4,42039	6,24395	
2,98975	2,60384	
13,06481	11,37132	
7,02204	2,85773	
15,64632	11,11263	
13,29572	10,89867	
12,30285	8,33732	
1,34358	0,80352	
3,15535	2,83482	
3,37188	5,17239	
1,42697	2,72136	
1,41518	1,84774	
0,00581	0,58627	
0,46617	0,17904	
0,03764	0,14229	
0,01230	0,01617	
0,38826	0,18683	
5,79609	4,25594	
4,92893	4,11052	
5,68704	4,47886	
1,13391	0,04062	
7,27719	2,49738	
0,09915	0,11530	
0,92030	0,65415	
1,20214	0,50689	
14,07357	10,98185	
4,76448	3,64516	
0,02129	0,16679	
2,39505	2,50230	
Vx	Vy	Vxy
1,21636	1,09843	1,08751
Sx	Sy	
1,10289	1,04806	



Estimations

r =	0,95499
b =	0,95029
a =	-0,01142

Residual standard deviation from the estimated points by regression

Sy:x = 0,445601667

yi estimated	residus	Smy:x
2,06951	-0,91900	0,31509
2,57681	-0,31772	
1,31702	1,42154	
2,25860	0,29629	
1,97578	0,07939	
2,33792	-0,03689	
3,47331	-0,07572	
3,16229	-0,04964	
3,28872	-0,03831	
2,94369	0,08921	
2,89646	0,07425	
2,96906	0,15353	
1,77195	0,22407	
2,76647	-0,29257	
3,83161	0,31891	
4,21753	-0,03667	
4,16456	0,02555	
1,56711	-0,32855	
3,72620	0,84185	
2,97193	0,18084	
2,27250	0,42582	
2,31900	0,22824	
2,72436	-0,22436	
3,01059	0,18295	
1,58135	-0,00828	
2,83661	0,11086	
2,21886	0,26474	
1,72391	0,37062	
2,14022	-0,18868	
2,58253	-0,23923	
2,02983	-0,31415	
1,67690	0,01321	
1,99592	-0,51880	
2,92277	0,18657	
1,95992	0,04008	
2,16644	-0,11407	
2,44921	0,06435	
2,22673	-0,10805	
2,25960	0,04143	
2,44797	0,14008	
2,30493	-0,53290	
2,20798	-0,46942	
2,71393	-0,73680	
2,50770	0,03054	
1,85212	-0,07397	
4,15631	0,06080	
2,38697	-0,00893	
4,62973	0,01274	
4,51905	-0,08332	
2,88193	0,26678	
1,30860	0,08047	
1,14836	0,00215	
1,16557	-0,16557	
2,77502	0,23280	
1,55704	0,44296	
3,68180	0,02122	
3,38867	0,01262	
1,48344	0,10460	
2,04601	0,18587	
1,85683	-0,07868	
1,61897	0,33257	
1,08190	0,30718	
1,52949	0,20907	
3,32171	0,05820	
2,47663	0,03216	
2,29623	0,12735	
1,95960	0,30433	
1,37431	-0,07328	
2,02840	-0,44035	
3,09479	-0,09389	
2,59097	-0,26617	
2,19781	-0,04910	
2,14005	-0,06879	
1,43368	-0,43368	
2,57810	0,03276	
3,44662	0,03872	
3,47709	0,09417	
1,35465	-0,35465	
3,92852	-0,02063	
5,19427	-0,04297	
4,54753	-0,58565	
5,42462	-0,30060	
5,21516	-0,11394	
5,09540	-0,28710	
1,98877	0,14486	
3,94657	0,00898	
4,00074	0,37088	
3,21760	0,71541	
3,56583	0,16204	
2,81266	-0,58704	
3,22141	-0,15624	
2,88995	0,14032	
2,71701	-0,01133	
3,18453	-0,11198	
4,38464	-0,15902	
4,25652	-0,05647	
1,16557	0,13546	
3,48238	-0,57506	
0,98491	0,66560	
2,59651	-0,02970	
3,41146	-0,07265	
3,50365	-0,23341	
5,28719	-0,17717	
4,23363	-0,11670	
2,86489	0,19028	
3,80600	0,07946	

Standard deviations of parameters

S(a)	0,08707	t(a)	0,13115	p(a=0)	0,89591
S(b)	0,02788	t(b)	1,78305	p(b=1)	0,07749

Repeatability
= 2,8 Sr

	Reference method	Alternative method
Sr	0,16411	0,14159
r	0,45950	0,39645
Rob.Sr	0,07538	0,08221
Rob.r	0,21106	0,23020

Bias

Differences	
-1,03928	
-0,46454	
1,34062	
0,16611	
-0,03599	
-0,17121	
-0,26943	
-0,22709	
-0,22238	
-0,07680	
-0,08929	
-0,01381	
0,11935	
-0,44931	
0,10645	
-0,26932	
-0,20433	
-0,42255	
0,63490	
0,01335	
0,29492	
0,09490	
-0,37890	
0,01344	
-0,10303	
-0,04955	
0,13665	
0,26841	
-0,31266	
-0,38635	
-0,43235	
-0,08653	
-0,63523	
0,02165	
-0,07447	
-0,23942	
-0,07579	
-0,23656	
-0,08879	
0,00000	
-0,66550	
-0,59695	
-0,89080	
-0,11266	
-0,18288	
-0,16865	
-0,14582	
-0,24147	
-0,33175	
0,10400	
0,00000	
-0,06994	
-0,23856	
0,07561	
0,34949	
-0,18340	
-0,17667	
0,01498	
0,06682	
-0,18783	
0,23586	
0,23856	
0,11704	
-0,12759	
-0,10942	
-0,00479	
0,18980	
-0,15720	
-0,55848	
-0,26781	
-0,41373	
-0,17609	
-0,19276	
-0,52070	
-0,11413	
-0,15360	
-0,09975	
-0,43753	
-0,23816	
-0,32672	
-0,83556	
-0,59640	
-0,39878	
-0,56567	
0,02891	
-0,20950	
0,14956	
0,53507	
-0,03651	
-0,74620	
-0,33679	
-0,02288	
-0,16548	
-0,29059	
-0,40041	
-0,29117	
0,06247	
-0,76926	
0,60206	
-0,17546	
-0,26313	
-0,42872	
-0,46578	
-0,35020	
0,02839	
-0,13167	
D = -0,15676	mean
D = -0,16706	median

APPENDIX C :

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.xylosus</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.scuri</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.xylosus</i>	Salad	black	no pink zone
<i>St.xylosus</i>	Offal	black	no pink zone
<i>Other genus</i>			
<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	/