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AFNOR Validation of Alternative Methods
FOOD MICROBIOLOGY

REPORT

*(Preliminary and Collaborative studies according to the
NF EN ISO 16140 standard)*

**ISO 16140 validation of the
3M™ Petrifilm™ Coliform count plates (CC)
for total coliforms enumeration with regard
to the reference method ISO 4832**

Quantitative method

This report includes 30 pages, with 3 annexes.
Only copies including the totality of this report are authorised.

PCC Total Coliforms Synthesis (Version 2)
September 3, 2008

*Cancel and replace the previous version which must be returned
to ADRIA Développement or destroyed internally*

ADRIA DEVELOPPEMENT

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The modifications brought to the report are indicated by a double line in the margin on the left.

Foreword

Competences of the laboratory are certified by COFRAC accreditation for the analysis marked with symbol♦.

Any element which allows analysis quality certification can be consulted by 3M SANTE.

The results are presented according to the NF EN ISO 16140 standard.

- ✓ **Firm:** 3M SANTE
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- ✓ **Expert laboratory:** ADRIA Développement
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- ✓ **Studied method:** ISO 16140 validation of the test 3M™ Petrifilm™
Coliforms (PCC) for total coliforms enumeration

- ✓ **Validation standard:** NF EN ISO 16140 (October 2003) : Food
microbiology – Protocol for the validation of
alternative methods

- ✓ **Standard method♦ :** ISO 4832: Horizontal method for the enumeration
of coliforms - Colony Count Technique

- ✓ **Validation area:** All food products, except raw shellfishes

♦ ISO 4832: analysis performed according to the COFRAC accreditation

1 INTRODUCTION

1.1 Validation standard

The validation study has been performed according to the NF EN ISO 16140 (October 2003) : Food microbiology – Protocol for the validation of alternative methods.

1.2 Protocol and principle of the alternative method

General use instructions are shown in Annex 1.

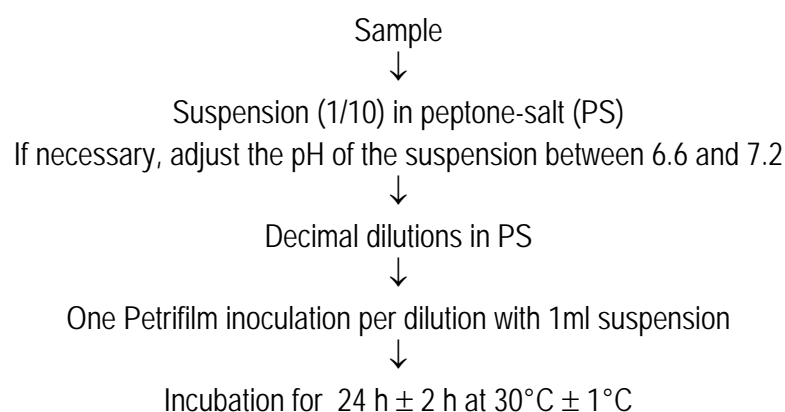
1.2.1 *Principle of the alternative method*

The Petrifilm CC plate is a ready-to-use system for the enumeration of the total coliforms. It is constituted by a cold water soluble dehydrated gel fixed between a support of polyethylene and a sheet leaf in polypropylene. The Petrifilm CC plate is based on the VRBL medium formulation.

After an incubation for 24 h \pm 2 h at 30°C \pm 1°C, coliforms appear as red colonies associated or not with gas

1.2.2 *Protocol*

The general protocol is presented below :

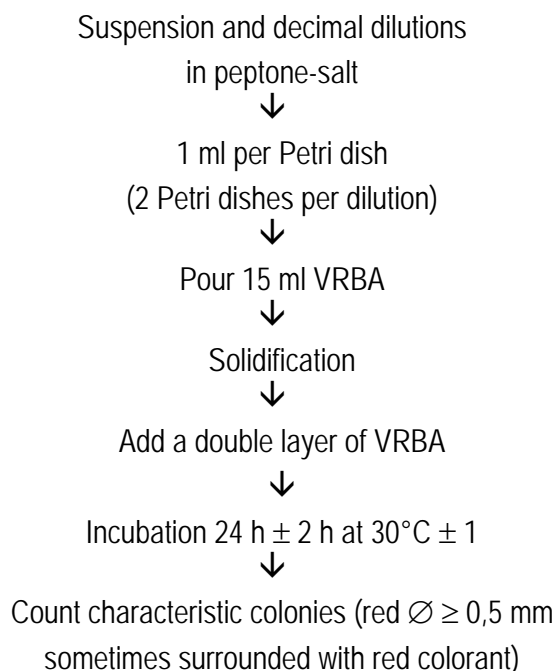


1.3 Application area

Food products, except raw shellfishes

1.4 Reference method

The reference method is the ISO 4832 method “Horizontal method for the enumeration of coliforms - Colony Count Technique”. The protocol is presented below:



2 COMPARATIVE STUDY

2.1 Bibliographical study

No papers related to the alternative method have been published since the last study which has been done in May 2002.

2.1 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.

2.1.1 Food matrices and protocols

The linearity was investigated with 5 couples (category / strain and matrix / category).

Five contamination levels were analysed in duplicate.

The contamination levels, the tested samples and the inoculated strains are presented in the table below :

Category	Strain
Ground beef	<i>Enterobacter cloacae</i> 58
Pasteurised milk	<i>Enterobacter sakazakii</i> 95
Egg product	<i>Klebsiella pneumoniae</i> 89
Raw fish fillet	<i>Escherichia coli</i> Ad 228
Green peas	<i>Escherichia coli</i> 19

2.1.2 Results

The bi-dimensional graphs are shown figure 1.

Figure 1 - Relative linearity: bi-dimensional graphs

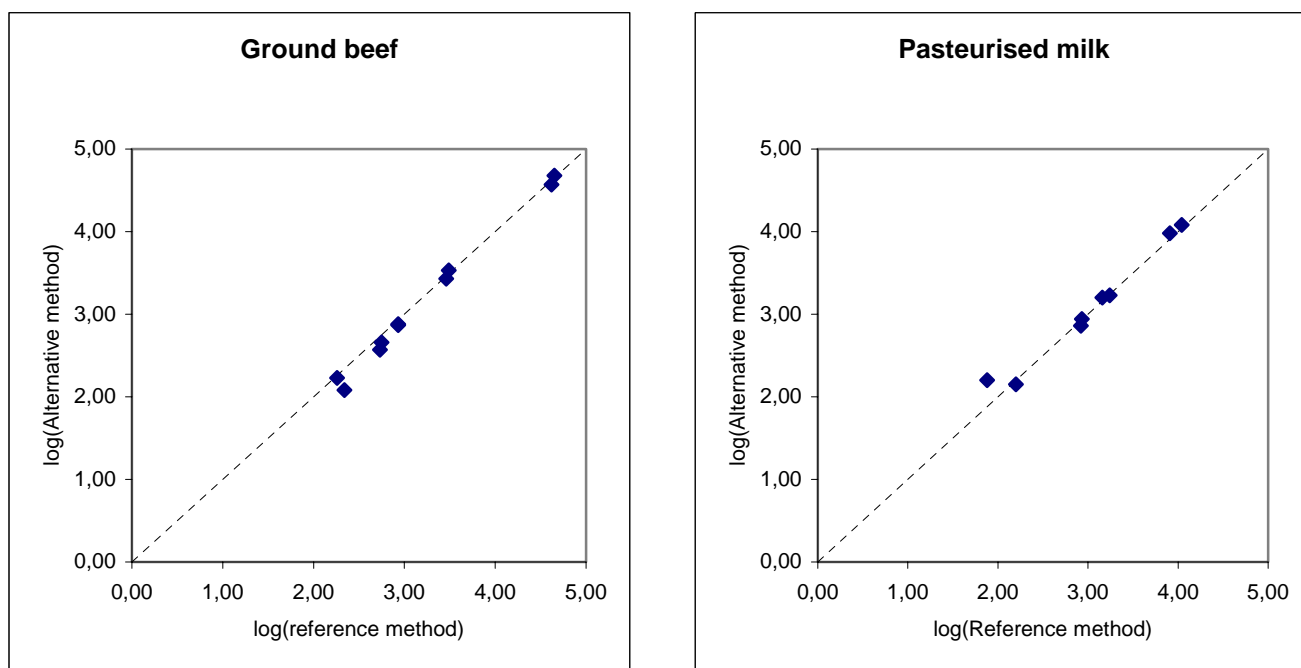
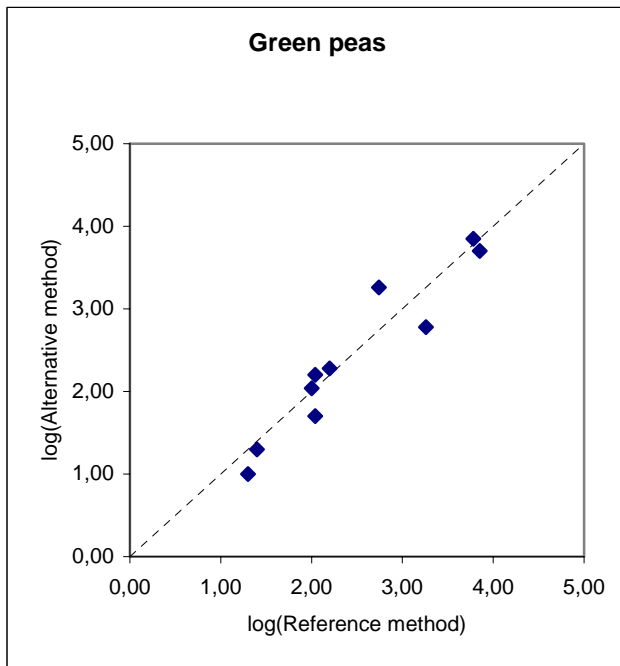
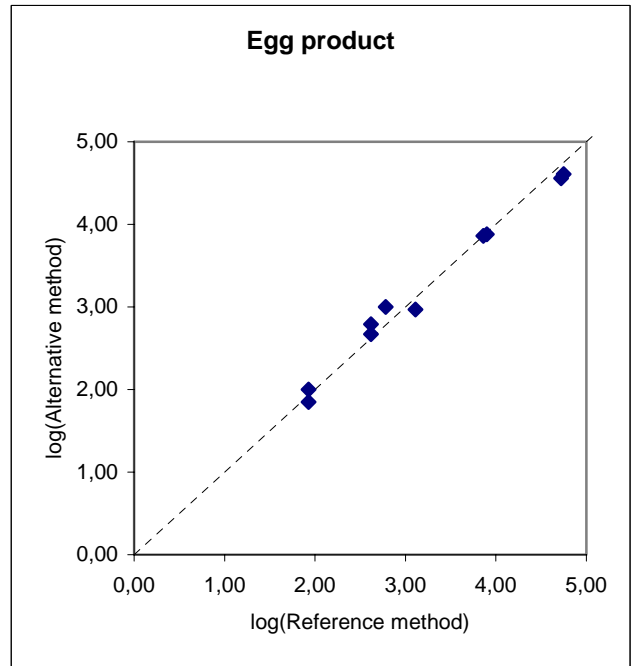
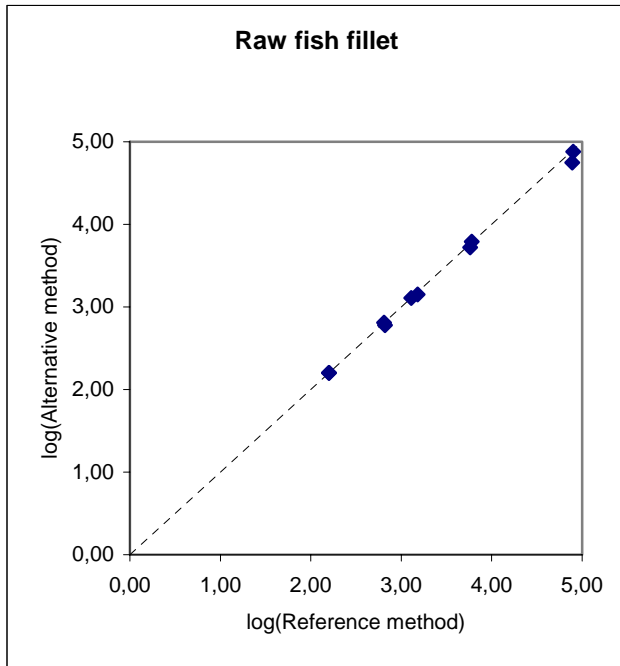


Figure 1 - Relative linearity: bi-dimensional graphs



2.1.3 Statistical interpretations

Table 1 - Comparison Petrifilm CC plate / ISO 4832

Matrix	R	Selected Regression	Rob.F	Critical value	P%	Correlation coefficient	Regression equation*
Ground beef	3,33	OLS1	0,69	5,41	60	0,997	log Alt = 1,060 log Ref. - 0,260
Pasteurised milk	0,63	GMFR	12,72	5,41	1	0,998	log Alt = 1,023 log Ref. - 0,012
Raw fish fillet	4,00	OLS1	3,53	5,41	10	0,999	log Alt = 0,973 log Ref. + 0,065
Egg product	1,67	GMFR	9,41	5,41	2	0,998	log Alt = 0,939 log Ref. + 0,194
Green peas	3,00	OLS1	0,642	5,41	62	0,980	log Alt = 1,056 log Ref. - 0,187

* x-axis and y-axis choice depends on the selected regression.

Statistical Interpretation :

P > 5 % : not significant

1 % < P < 5 % : significant

P < 1 % : highly significant

The regression lines are shown in Annex 2.

2.1.4 Conclusion

The correlation coefficients are all above 0,99 whatever the matrix tested.

The non-linearity test is not significant for the matrices beef meat, raw fish and beans.

The correlation coefficients associated with P value below 5 % show high values which decrease the non-linearity test robustness.

The Petrifilm CC plate shows satisfying linearity.

2.2 Relative accuracy

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

The bias is the difference between the expectation of the test results and an accepted reference value.

2.2.1 Number and nature of the samples

Categories and types of analysed food are presented in table 2.

Table 2 – Number and nature of the samples

Categories	Types	Number of analysed samples	Number of results used in statistical tests
Meat products	Raw meats, raw and cooked delicatessen, ready-to-eat food	67	49
Milk products	Raw milk, cheeses, creams, ice creams	51	28
Egg products and pastries	Egg product, pastries, mayonnaises	26	17
Vegetables	Frozen vegetables, salads, spices, cooked vegetables, flours	41	30
Sea food	Fishes, cured products, ready-to-eat food	42	23
	All products	227	147

Only contaminated samples have been analysed.

2.2.2 Results

Samples have been analysed in duplicate for both methods.

Table 3 - Petrifilm CC plate / ISO 4832

Food category	Contamination scale (log CFU/g)
Meat products	1,00 to 7,70
Milk products	1,00 to 7,56
Egg products and pastries	1,30 to 6,57
Vegetables	1,00 to 6,15
Seafood	1,30 to 5,76

Bi-dimensional graphs for each category and for all samples analysed are presented figure 2.

Figure 2 – Relative accuracy : bi-dimensional graphs

By category

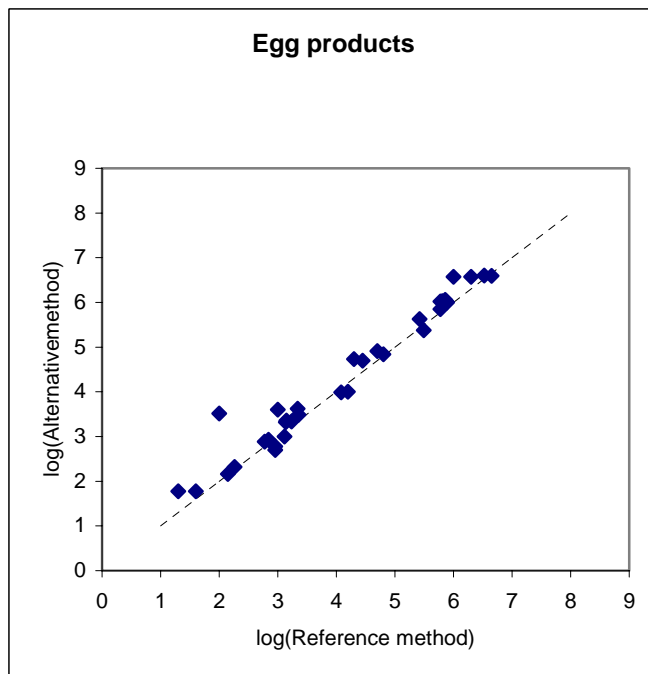
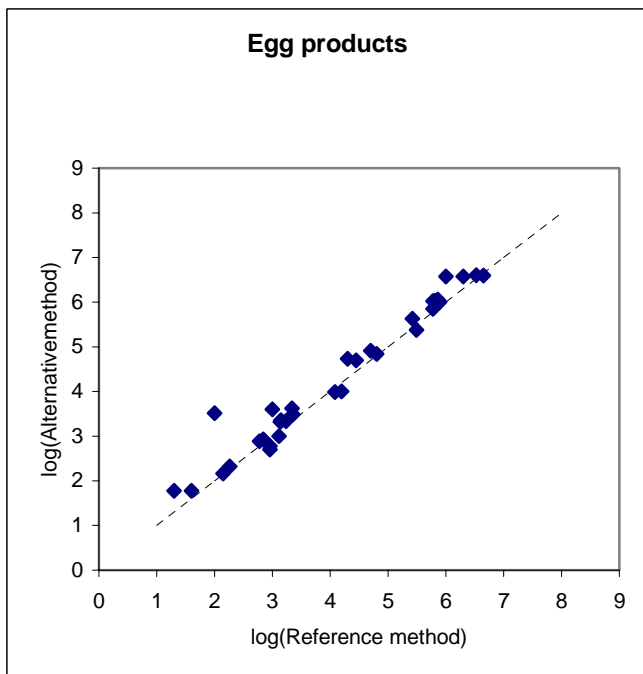
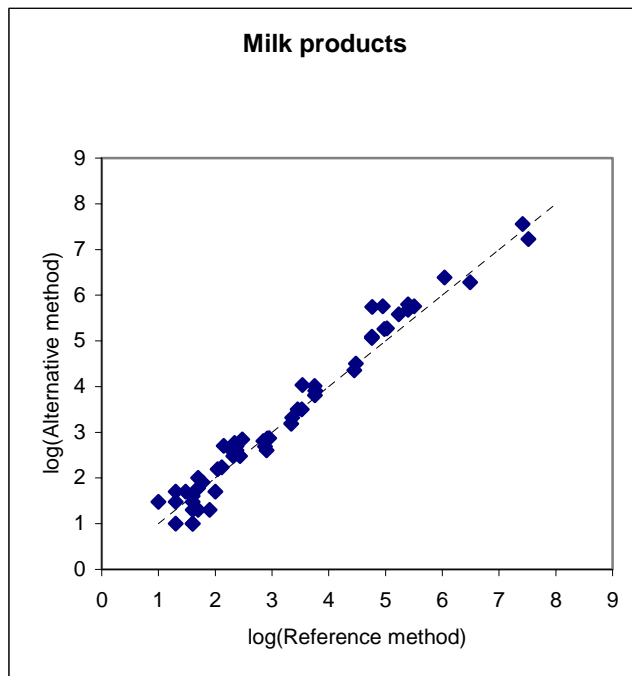
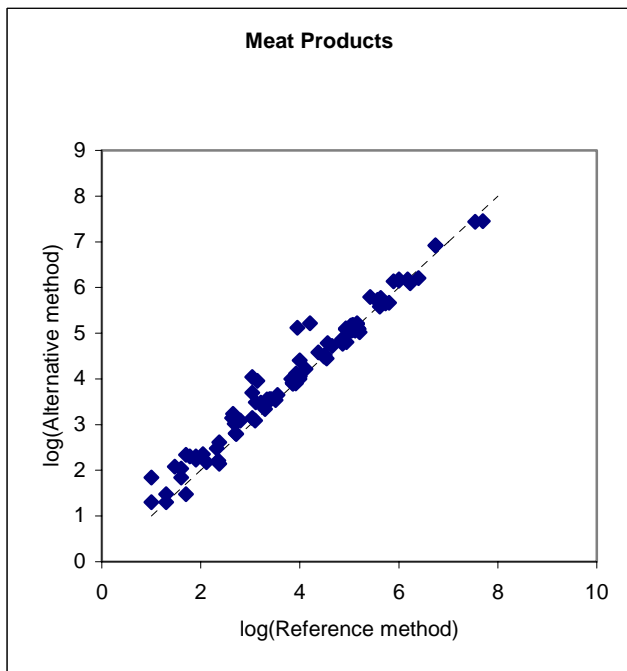
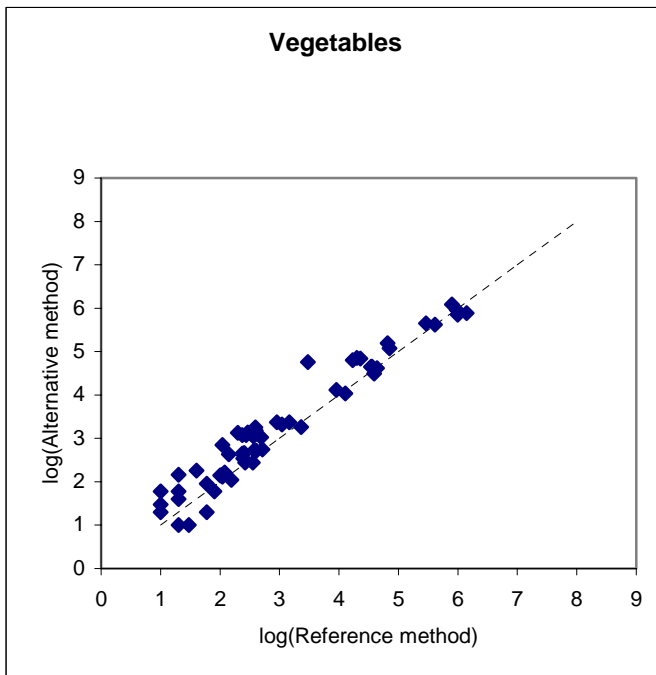
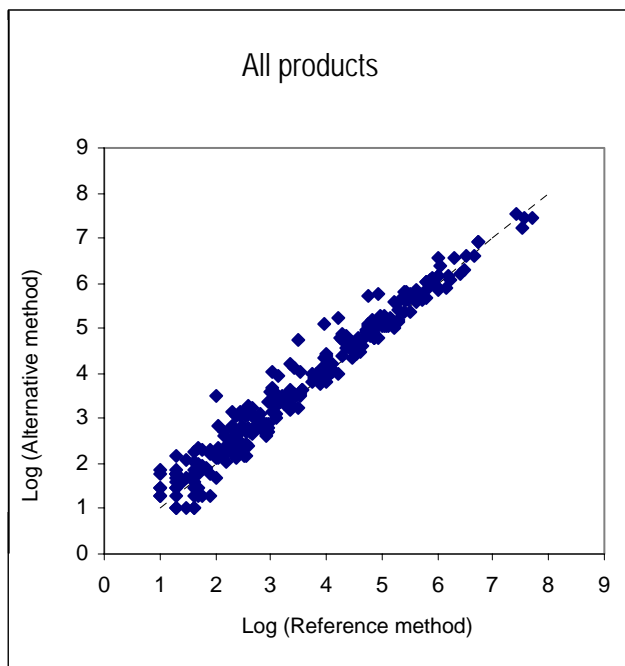


Figure 2 – Relative accuracy : bi-dimensional graphs



All products



2.2.3 Statistical interpretations

Regression lines (graph and equation representations) for each food category and for all matrices are presented in Annex 3

Table 4 - Petrifilm CC plate / ISO 4832

Categories	n	R	Selected regression	a	t(a)	b	t(b)	Critical T	P%	
									Ordinate at 0	Slope at 1
Meat products	49	0,70	GMFR	0,404	4,274	0,940	2,730	2,012	0	1
Milk products	28	1,06	GMFR	- 0,097	0,947	1,062	2,162	2,056	35	4
Egg products and pastries	17	0,54	GMFR	0,217	1,087	0,990	0,215	2,131	29	83
Vegetables	30	0,54	GMFR	0,347	2,708	0,974	0,678	2,048	1	50
Seafood	23	0,76	GMFR	0,140	0,835	0,992	0,187	2,080	41	85

Statistical Interpretation :

P > 5 % : not significant

1 % < P < 5 % : significant

P < 1 % : highly significant

Category	Bias D	Alternative method repeatability limit	Reference method repeatability limit
Meat products	0,119	0,166	0,232
Milk products	0,094	0,336	0,309
Egg products and pastries	0,156	0,146	0,268
Vegetables	0,155	0,224	0,406
Seafood	0,042	0,160	0,205
All products	0,101	0,188	0,232

2.2.4 Conclusion

Bias between both methods show low values whatever the categories and vary between 0,042 to 0,156 log CFU/g.

The alternative method shows generally better repeatability limit values than the reference method.

The regression equations are the following :

- **Meat products:** Log (reference method) = 0,940 Log (alternative method) + 0,404
- **Milk products:** Log (reference method) = 1,062 Log (alternative method) – 0,097
- **Egg products and pastries:** Log (reference method) = 0,990 Log (alternative method) + 0,217
- **Vegetables:** Log (reference method) = 0,974 Log (alternative method) + 0,347
- **Seafood:** Log (reference method) = 0,992 Log (alternative method) + 0,140

For all products, the regression equation is:

$$\text{Log (reference method)} = 0,987 \text{ Log (alternative method)} + 0,209$$

The Petrifilm CC plate shows satisfying relative accuracy for all products.

2.3 Detection limit (LOD) and quantification limit (LOQ)

The critical level is defined as the smallest amount which can be detected (not null), but not quantified as an exact value. Below this value, it cannot be sure that the true value is not null.

The detection limit is defined as being higher than the critical level because it involves a power, the probability $1-\beta$, which has to be well over 50 %, for example 95 %.

The quantification limit is defined as the smallest amount of analyte (that is the lowest actual number of organisms) which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation.

2.3.1 Protocol

The detection limit of the alternative method has been done with pure culture of *Escherichia coli*. Three different levels of inoculation have been tested, with six replicates per level, i.e. a total of 18 analyses by the alternative method.

Quantification limit has been calculated for six independent determinations done with sterile dilution solution.

2.3.2 Results

Data are intrinsic to the method used and are presented in the following tables :

Table 5 - Petrifilm CC plate

Level	Positive samples nb	Standard deviation	Bias
0,5	3/6	0,816	0,5
1	3/6	1,549	0,5
5	6/6	1,602	2

	Formulas	Obtained values
LC	$S_0 + X_0$	1,8
LOD	$S_0 + X_0$	3,2
LOQ	$S_0 + X_0$	8,7

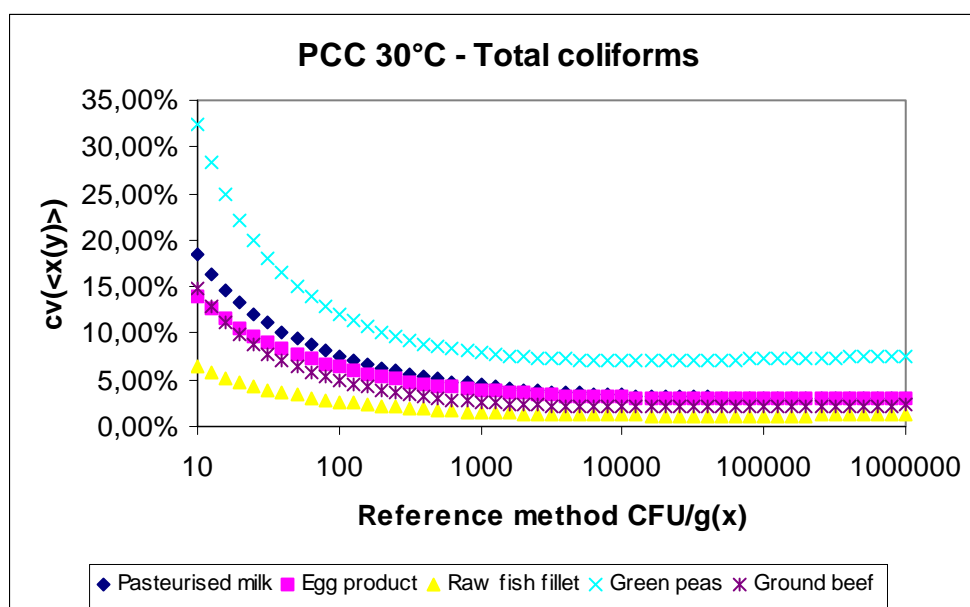
2.4 Relative Sensitivity

The relative sensitivity is defined as the ability of the alternative method to detect two different amounts of analyte measured by the reference method within a given matrix, at a specified average value, or over the whole measurement range ; that is, it is the minimal quantity variation (increase of the analyte concentration x) which gives a significant variation of the measured signal (response y).

Data are intrinsic to the method used and are obtained from the results of the linearity study.

Accuracy patterns obtained for different matrices are presented figure 3.

Figure 3 - Accuracy patterns for the different matrices used



2.5 Specificity

The specificity is defined as the degree to which a method is affected (or not) by the other components present in a multi-component sample. That is the ability of a method to measure exactly a given analyte, or its amount, within the sample without interference from non-target components such as a matrix effect, or background noise.

Five presumed target strains and four non-target strains have been tested in duplicate by the alternative and reference methods, in order to complete data obtained in 1997.

The five target strains give similar results on Petrifilm CC plate and VRBA. For the non-target strains, two *Providencia* strains grow on VRBA and give microcolonies on Petrifilm CC plate.

The specificity and the selectivity of both methods are similar.

2.6 Practicability

The saving of time is mainly obtained by the fact that:

- the Petrifilm CC plate is ready-to-use and allows to avoid the medium preparation.
- it is not necessary to pour a double layer of medium.

The time delays to obtain results are equivalent between both method, i.e. $24 \text{ h} \pm 2 \text{ h}$. The space needed for incubation is much smaller for the Petrifilm CC plate than for ISO 4832 method. The Petrifilm CC plate minimizes the volume of wastes and the manipulation between two different analysis steps.

3 COLLABORATIVE STUDY

3.1 Organisation study

✓ *Collaborator laboratories*

14 laboratories have participated to this study.

✓ *Instructions for collaborator laboratories*

Detailed instructions have been transmitted to the collaborators by the expert laboratory.

✓ *Samples*

Pasteurised semi-skimmed milk has been inoculated by *Escherichia coli* 94, isolated from dairy product.

✓ **Inoculation**

Inoculation levels targeted are :

- 0 CFU/ml,
- 10 – 100 CFU/ml,
- 100 – 1 000 CFU/ml,
- 1 000 – 10 000 CFU/ml.

Each laboratory received eight flasks of 25 ml sample, i.e. two flasks per inoculation level. Furthermore, one non-inoculated sample have been added to the package for total viable count microflora (NF ISO 4833 method).

✓ **Labelling and shipping**

Coded samples (code is only known by the expert laboratory) have been placed in isothermal boxes which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing temperature register has been added to the package in order to register temperature profile during transport and at reception.

Samples have been shipped in 24 h to laboratories of the collaborative study. Sample temperature should be lower or equal to 8°C during transport, and between 0°C - 8°C at arrival.

✓ **Analyses**

Collaborative study laboratories and the expert laboratory have carried out the analyses with the alternative and reference methods.

A stability study of the strain inoculated has been realised in order to verify there is no evolution during the transport.

3.2 Results

3.2.1 Results obtained by the expert laboratory

✓ Strain stability during transport

In order to evaluate the *Escherichia coli* 94 strain variability during transport, bacterial count of all inoculated flasks have been checked at different time, i.e. inoculation time, after 24 h and 48 h of conservation at 2°C. Results are reported in table 6.

Table 6 - *Escherichia coli* 94 count with the alternative method and reference method (in log CFU/ml)

	Level 1		Level 2 2		Level 3	
	Reference method	Atlernative method	Reference method	Atlernative method	Reference method	Atlernative method
Day 0	73 / 85	55 / 80	720 / 670	450 / 420	6 600 / 6 800	3 500 / 4 900
Day 1	47 / 42	61 / 64	630 / 550	480 / 520	7 000 / 5 700	3 500 / 3 500
Day 2	83 / 91	74 / 68	740 / 610	650 / 810	6 500 / 1 600	4 900 / 6 200

No evolution of the strain has been observed after 48 h of storage at 4°C in the isothermal box.

✓ Results obtained for both methods

The mesophilic aerobic microflora has been done on the matrix with ISO 4833 method. The results varied from 70 to 3 600 CFU/ml.

The results obtained by the expert laboratory for *Escherichia coli* count for the both method are given table 7.

Table 7 - Expert laboratory results (in log CFU/g)

Targeted rate (log CFU/g)	Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
0	0	0	0	0
1 to 2	1,67	1,62	1,79	1,81
2 to 3	2,80	2,74	2,68	2,72
3 to 4	3,85	3,76	3,54	3,54

Contamination levels targeted have been reached.

3.2.2 Results obtained by the collaborative laboratories

3.2.2.1 Sample temperature on receipt

Measured temperatures on receipt are listed table 8.

The packages have been chipped in 24 h for the 14 laboratories. Any temperature upper than 8°C has been noted at reception.

Table 8 – Sample temperature on receipt

Laboratories	Temperature measured by the thermobutton (°C)
A	2,2 (J2)
B	2,0
C	5,3
D	1,7
E	2,7
F	3,3
G	2,8
H	4,9
I	3,5
J	1,5
K	1,8
L	3,9
N	2,5
O	5,0

3.2.2.2 Sample temperature during transport

Any temperature upper than 8°C has been noted during transport.

3.2.2.3 Results

The laboratory A has received the package at J1, but has realised the analyses only at J2 and the laboratory I has not realise two successive dilutions. These results were not took into account for statistical interpretation.

The results from 12 laboratories have been retained for statistical interpretation.

A results synthesis is presented in table 9.

Table 9 - Results synthesis obtained by ISO 4832 method and Petrifilm CC plate

Laboratories	Level 0				Level 1				Level 2				Level 3			
	Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method		Reference method		Alternative method	
A	0	0	0	0	84	65	60	53	800	600	400	360	9300	5500	5000	3800
B	0	0	0	0	64	63	75	63	690	710	560	650	4600	6500	4200	4700
C	0	0	0	0	38	33	55	57	460	430	540	410	4800	3600	3100	4000
D	0	0	0	0	74	62	85	64	720	690	560	660	5700	6600	3200	3900
E	0	0	0	0	73	84	76	68	620	960	670	650	8700	7700	6900	6200
F	0	0	0	0	66	63	64	76	560	560	550	560	7000	5300	5500	4700
G	0	0	0	0	61	73	77	91	820	750	710	620	7800	9100	6600	6100
H	0	0	0	0	62	73	71	76	690	680	560	560	6500	6700	4200	5100
J	0	0	0	0	56	65	62	44	590	850	490	500	4900	5900	6500	4000
K	0	0	0	0	77	67	76	68	770	750	660	660	5700	5900	5100	5500
L	0	0	0	0	21	33	70	65	380	540	540	600	6400	5900	4800	5300
N	0	0	0	0	76	88	68	87	860	920	680	650	9500	9900	8000	5200
O	0	0	0	0	92	86	77	89	900	880	650	670	7800	6500	5800	5000

3.2.2.4 Statistical interpretation

↳ Bias calculation

For each level, difference between duplicate means (d_i) obtained by the alternative method and reference method has been calculated as : $d_i = (M_{i,alt} - M_{i,ref})$.

Median ($MED\{d_i\}$) of d_i allows the determination of bias D ($MED\{d_i\} = \text{bias D}$).

Bias D and robust standard deviation $S\{d_i\} = K1S_n$ give t statistic ($t(d) = MED\{d_i\} \sqrt{n} / S\{d_i\}$). This t value obtained is compared to a critical value found in Student table (for $n = 12$, critical $t = 2,201$).

t(d) values obtained by level are reported in table 10.

Table 10 - t(d) values obtained by level

Level	Bias D	t(d)	Critical t ddl (n-1)	Conclusion
1 (n = 12)	+ 0,03	1,525	2,201	Biases D Non significant bias
2 (n = 12)	- 0,07	3,137	2,201	Significant bias
3 (n = 12)	- 0,09	5,814	2,201	Significant bias f

Critical level : t(d) < critical t

↪ **Repeatability calculation**

Values obtained for the repeatability limit, as well as values obtained for F test are reported in table 11.

Table 11 – Obtained values for repeatability limit and values for F test

Level	Repeatability limit		Calculated F (or 1/F*)	Critical F (0,05 ; n ; n)	P %
	Reference method	Alternative method			
1 (n = 12)	0,183	0,199	1,175	2,69	39
2 (n = 12)	0,070	0,048	2,127	2,69	10
3 (n = 12)	0,192	0,195	1,033	2,69	47,8

Statistical Interpretation

P > 5% : non significant

0,1 % < P < 1 % : highly significant

1 % < P < 5 % : significant

P < 0,1 % : very highly significant

↪ **Reproducibility**

Values obtained for reproducibility limit, as well as values obtained for F test are given in table 12.

Table 12 - Obtained values for reproducibility limit and F Test values

Level	Reproducibility limit		Calculated F (or 1/F*)	Critical F (0,05 ; n - 1 ; n - 1)	P %
	Reference method	Alternative method			
1 (n = 12)	0,243	0,180	1,820	2,82	17
2 (n = 12)	0,196	0,143	1,865	2,82	16
3 (n = 12)	0,305	0,300	1,028	2,82	48

Statistical Interpretation

P > 5% : non significant

1 % < P < 5 % : significant

0,1 % < P < 1 % : highly significant

P < 0,1 % : very highly significant

↪ Ratio reproducibility limit / repeatability limit

The ratio reproducibility limit / repeatability limit are given in table 13.

Table 13 - Ratio of reproducibility limit / repeatability limit

Level	Reference method	Alternative method
1 (n = 12)	1,321	0,906
2 (n = 12)	2,793	2,983
3 (n = 12)	1,588	1,542

Critical level : reproducibility / repeatability < 2**↪ Dispersion between laboratories**

Level	Reference method F	Alternative method F ou 1/F	Critical F (0,05 ; n-1 ; n)
1 (n = 12)	2,508	1,563	2,79
2 (n = 12)	14,600	16,791	2,79
3 (n = 12)	4,046	3,753	2,79

Niveau critique : F ou 1/F < Fcritique

Discussion

The bias between the reference method and the Petrifilm CC plate is not significant for the first level. A significant bias is obtained for the two other levels.

However, the observed values (respectively - 0,07 a, d - 0,09 log CFU/g) are low values.

The repeatability and reproducibility limits are similar for both methods for the tested levels.

The ratio repeatability / reproducibility are similar for both methods. They are above 2 for level 2.

The dispersion between the laboratories is important for level 2 for both methods ; it is better for the alternative method for levels 1 and 3.

4 CONCLUSION

Conclusions of comparative study are the following:

The Petrifilm CC plate shows satisfying linearity.

The Petrifilm CC plate shows satisfying relative accuracy for all products.

Petrifilm CC plate and ISO 4832 standard show equivalent specificity and selectivity.

The time delays to obtain results are equivalent between both method, i.e. 24 h \pm 2 h. The Petrifilm CC plate minimizes the incubation space, the volume of wastes and the manipulation between two different analysis steps.

Conclusions of the interlaboratory study are the following:

The bias between the two methods are small for the three level tested : + 0,03, - 0,07 et - 0,09 CFU/g.

The repeatability and the reproducibility limits are similar between both methods.

Annex 1 - Alternative method use instruction

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3M™ Petrifilm™

(English)

Coliform Count Plate

DESCRIPTION

The 3M™ Petrifilm™ Coliform Count (CC) Plate is a sample-ready-culture-medium system which contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. Petrifilm CC plates are used for the enumeration of coliforms in the food and beverage industries. Petrifilm CC plate components are decontaminated though not sterilized. 3M Microbiology is certified to ISO (International Standards Organization) 9001.

AOAC INTERNATIONAL and the U.S. FDA Bacteriological Analytical Manual (BAM) define coliforms as gram-negative rods, which produce acid and gas from lactose fermentation. Coliform colonies growing on the Petrifilm CC plate produce acid, which causes the pH indicator to deepen the gel color; gas trapped around red colonies indicates coliforms.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832, enumerating coliforms by colony-count technique, defines coliforms as acid producers on VRB with lactose (VRBL) agar. On Petrifilm CC plates these acid-producing coliforms are indicated by red colonies with or without gas production. ISO method 4831, enumerating coliforms by the most probable number (MPN) method, defines coliforms by their ability to grow and produce gas from lactose in a selective broth. On Petrifilm CC plates these coliforms are indicated by red colonies associated with gas. AFNOR has validated Petrifilm CC plate in comparison to ISO method 4831 and ISO method 4832 for enumeration of total coliforms. AFNOR has also validated Petrifilm CC plate in comparison to NF V08-017, for enumeration of thermotolerant coliforms.

CAUTIONS

3M has not documented Petrifilm CC plates for use in industries other than food and beverage. For example, 3M has not documented Petrifilm CC plates for testing water, pharmaceuticals or cosmetics.

Petrifilm CC plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of coliform or other bacteria.

The Petrifilm CC plates have been evaluated following AOAC / AFNOR / NordVal guidelines and met validation criteria in representative samples of the following food categories: vegetables, meat, poultry, seafood, dairy, and processed foods.

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Do not use Petrifilm CC plates in the diagnosis of conditions in humans or animals.

For information on documentation of product performance contact your official 3M Microbiology representative.

USER RESPONSIBILITY

No one culture medium will always recover the exact same strains or enumerate a particular strain exactly as does another medium. In addition, external factors such as sampling methods, testing protocols, preparation time and handling may influence recovery and enumeration. The food sample itself may influence results. For example, foods with high sugar content may increase the potential for gas production from non-coliform Enterobacteriaceae.

It is the user's responsibility in selecting any test method to evaluate a sufficient number of samples with particular foods and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' or suppliers' requirements.

As with any culture medium, Petrifilm CC plate results do not constitute a guarantee of quality of food or beverage products or processes that are tested with the plates.

The user must train its personnel in proper testing techniques: for example, Good Laboratory Practices (U.S. Food and Drug Administration, Title 21, Part 58 of the Code of Federal Regulations) or ISO 17025.

DISCLAIMER OF WARRANTIES / LIMITED REMEDY

UNLESS OTHERWISE PROHIBITED BY LAW, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Petrifilm plate is proven to be defective, 3M or its authorized distributor will replace or, at its option, refund the purchase price of any plate. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defect in a product and return the product to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Microbiology representative for a Returned Goods Authorization.

LIMITATION OF 3M LIABILITY

UNLESS OTHERWISE PROHIBITED BY LAW, 3M WILL NOT BE LIABLE TO USER OR OTHERS FOR ANY LOSS OR DAMAGE, WHETHER DIRECT, INDIRECT, SPECIAL,

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INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING, BUT NOT LIMITED TO, LOST PROFITS. Except where prohibited by law, in no event shall 3M's liability under any legal theory exceed the purchase price of the plates alleged to be defective. Customer may have additional rights and should seek advice in country of purchase.

STORAGE AND DISPOSAL

Store unopened Petrifilm plate pouches refrigerated or frozen at temperatures $\leq 8^{\circ}\text{C}$ (46°F). Just prior to use, allow unopened pouches to come to room temperature before opening. 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. It is recommended that resealed pouches of Petrifilm plates be stored in a freezer (see below) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 50% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place Petrifilm plates in a sealable container. To remove frozen Petrifilm plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. Plates should not be used past their expiration date. The freezer that is used for open pouch storage must not have an automatic defrost cycle as this would repeatedly expose the plates to moisture which can damage the plates.

Do not use plates that show discoloration. Expiration date and lot number are noted on each package of Petrifilm plates. The lot number is also noted on individual plates.

After use, Petrifilm CC plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.

INSTRUCTIONS FOR USE

Sample Preparation

1. Use appropriate sterile diluents:

Butterfield's phosphate buffer² 2 0.1% peptone water, peptone salt diluent,³ buffered peptone water,³ saline solution (0.85-0.90%), bisulfite-free letheen broth or distilled water.

Do not use diluents containing citrate, bisulfite or thiosulfate with Petrifilm plates; they can inhibit growth. If citrate buffer is indicated in the standard procedure, substitute with one of the buffers listed above, warmed to $40\text{--}45^{\circ}\text{C}$.

AFNOR validated methods:

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Only ISO diluents listed above and referenced are in the scope of the validation.

2. Blend or homogenize sample.
3. For optimal growth and recovery of microorganisms, adjust the pH of the sample suspension to 6.6 - 7.2. For acidic products, adjust the pH with 1N NaOH. For alkaline products, adjust the pH with 1N HCl.

Plating

1. Place the Petrifilm CC plate on a flat, level surface (see figure a).
2. Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension onto the center of bottom film (see figure b).
3. Roll the top film down onto the sample to prevent trapping air bubbles (see figure c).
4. Place the plastic spreader with the flat side down on the center of the plate (see figure d). Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire Petrifilm plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate plates in a horizontal position with the clear side up in stacks of no more than 20 plates. Several incubation times and temperatures can be used depending on current local reference methods.

For example:

AOAC® Official Methods™ (986.33 Bacteria and Coliform Counts in Milk, Dry Rehydratable Film Methods and 989.10 Bacterial and Coliforms Counts in Dairy Products, Dry Rehydratable Film Methods)

Incubate Petrifilm CC plates 24 h \pm 2 h at $32^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

AOAC® Official Methods™ (991.14 Coliform and Escherichia coli Counts in Foods, Dry Rehydratable Film Methods)

Incubate Petrifilm CC plates 24 h \pm 2 h at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

AFNOR validated methods

Total coliforms: in comparison to ISO 4832 (3M-01/2-09/89 A) and in comparison to ISO 4831 (3M-01/2-09/89 B)

All food except raw shellfish.

- Incubate Petrifilm CC plates 24 h \pm 2 h at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ or $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

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- As recommended in ISO 4831 and ISO 4832, these temperatures should be agreed upon between concerned parties and mentioned in the analysis certificate.

Thermotolerant coliforms: in comparison to NF V08-060, VRBL incubated at $44.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (3M-01/2-09/89 C).
All food.

- Incubate Petrifilm CC plates $24\text{ h} \pm 2\text{ h}$ at $44^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Nordic System for validation of alternative microbiological methods, NordVal Validation (Ref. No.: 2003-20-5408-00011)

Refer to NordVal validation for Petrifilm CC plate method details.

Interpretation

1. Petrifilm CC plates can be counted using a standard colony counter or other illuminated magnifier.
Gas produced by coliform may disrupt the colony so that the colony "outlines" the bubble. This should be counted as a single coliform. Do not count colonies on the foam dam since they are removed from the selective influence of the medium. Do not count artifact bubbles that may be present.

The interpretation of coliform colonies on the Petrifilm CC plate varies by method. For example:

AOAC Official Methods

Coliform colonies are red and closely associated (within one colony diameter) with entrapped gas. Colonies not associated with gas (a distance greater than one colony diameter between colony and gas bubble) are not counted as coliforms.

OR AFNOR validated methods

- As compared to ISO method 4831 (MPN method), coliform colonies are red and closely associated (within one colony diameter) with entrapped gas (see figure e).

Note: a high ratio of non gas-producing gram-negative rods may require a higher dilution in order to enumerate gas producing coliforms within the recommended counting range.

- As compared to ISO method 4832 and NF V08-060 (VRBL methods), count all red colonies with or without gas.

2. The circular growth area is approximately 20 cm^2 . Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate (see figure f); estimations are outside of the AFNOR validation scope.

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3. When present in large numbers, Petrifilm CC plates may have a deepening of the gel color and either or both of the following characteristics: many small, indistinct colonies and/or many gas bubbles. High concentrations of coliforms will cause the growth area to turn dark red (see figure g). When this occurs, record results as too numerous to count (TNTC). When an actual count is required, plate at a higher dilution.
4. Where necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel (see figure h). Test using standard procedures.
5. If the plates cannot be counted within 1 hour of removal from the incubator, they may be stored for later enumeration by freezing in a sealable container at temperatures \leq minus 15°C for no longer than one week.

For further information refer to the appropriate Petrifilm plate "Interpretation Guide." If you have questions about specific applications or procedures, please contact your official 3M Microbiology representative nearest you.

References

1. FDA. 1998. Bacteriological Analytical Manual, 8th ed., Revision A, Appendix 3.64.
2. International Standards Organization, ISO 6887-1:1999. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination
3. International Standards Organization, ISO 8261:2001. Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination

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Explanation of symbols



- Attention, see instructions for use



- The lot in a box and the hourglass symbols are symbols that represent lot number and expiration date. The hourglass is followed by a year and month which represents the expiration date (year and month: 2010-10). The entire line after the hourglass represents the lot number. (2010 - 10 AZ).

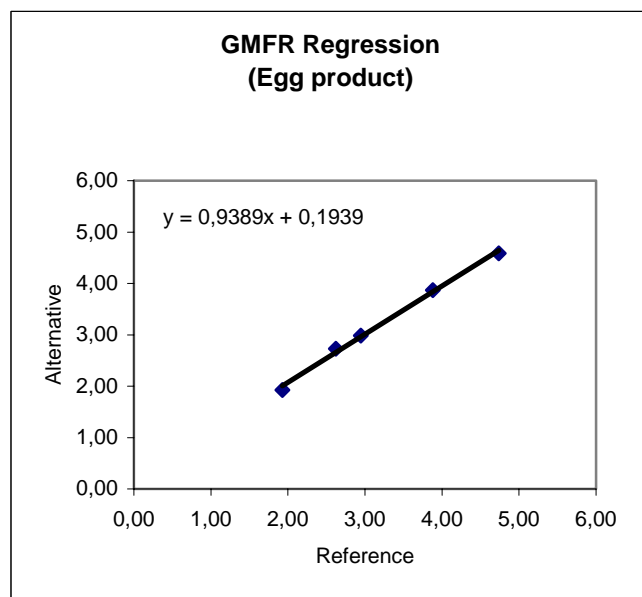
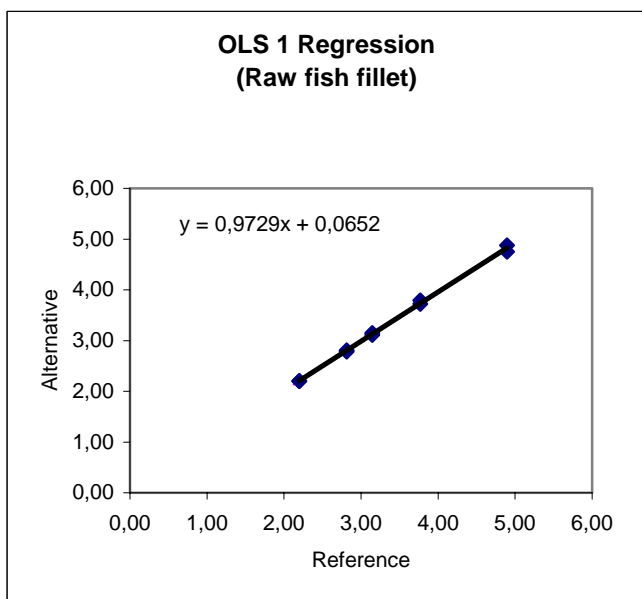
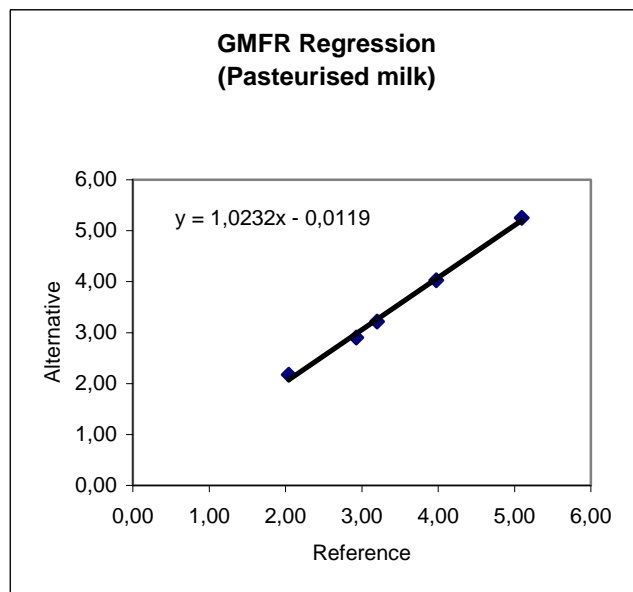
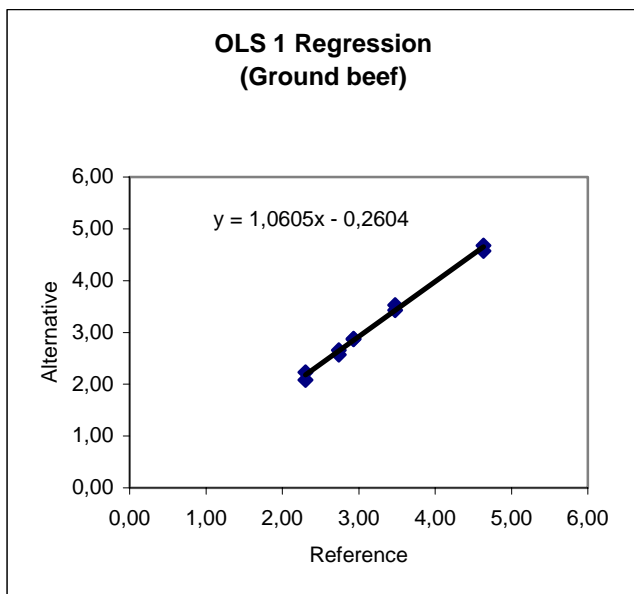


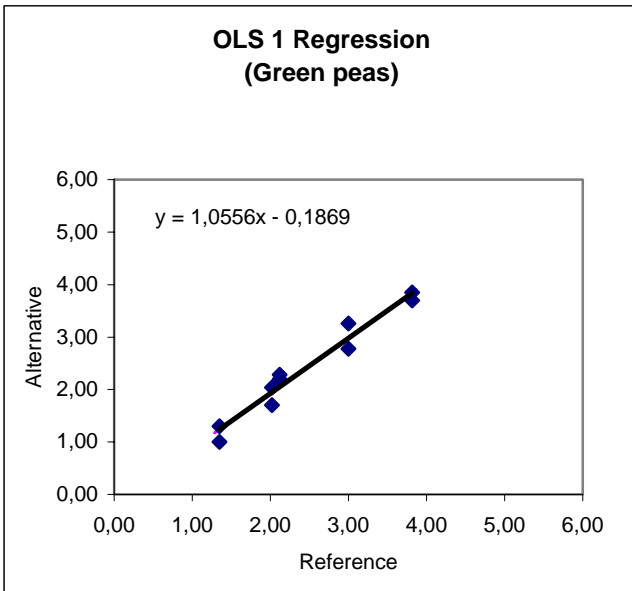
- Store below given temperature.

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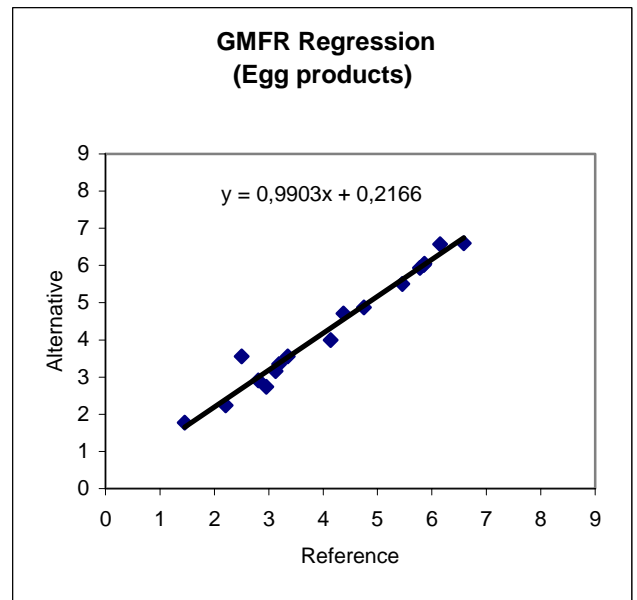
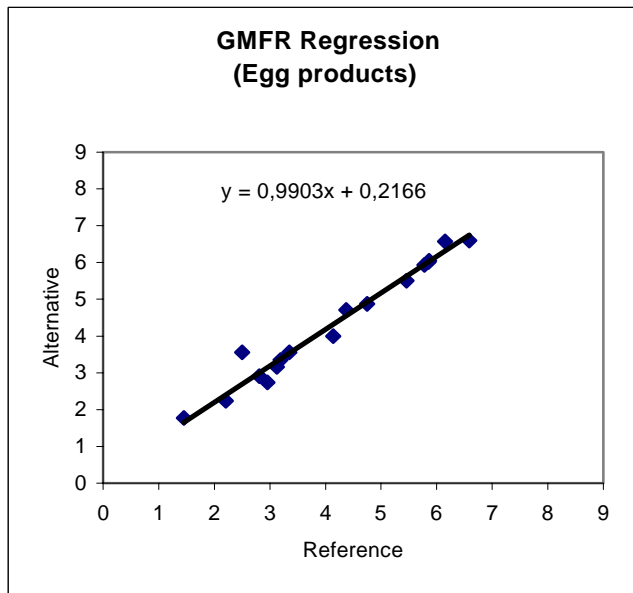
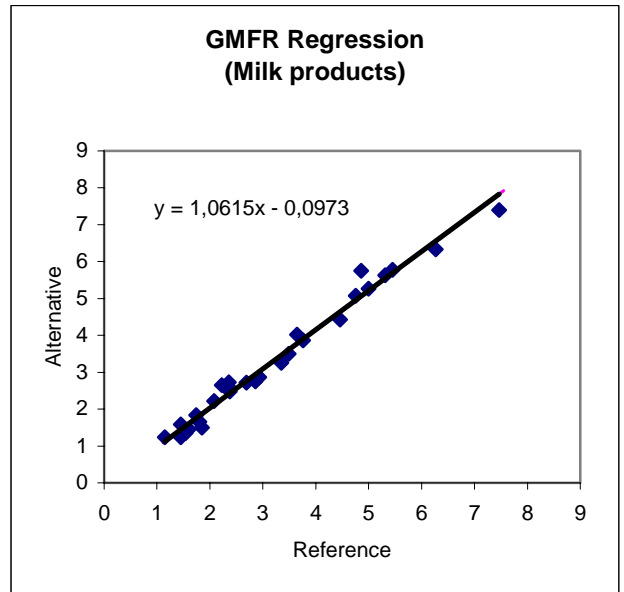
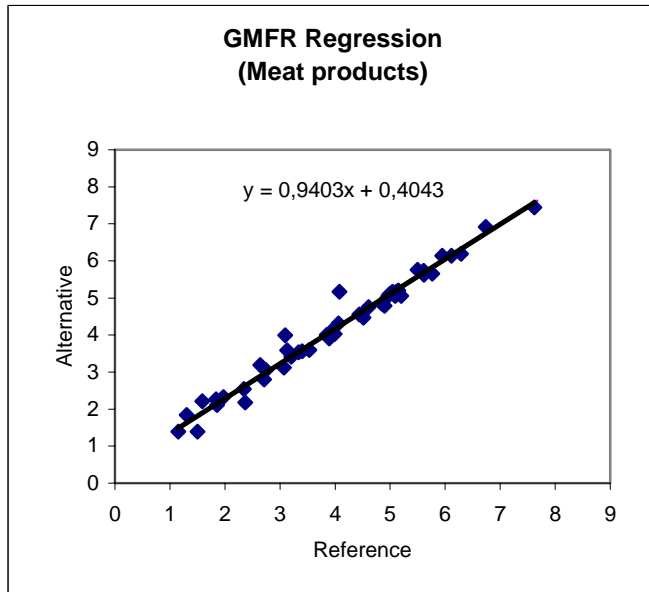
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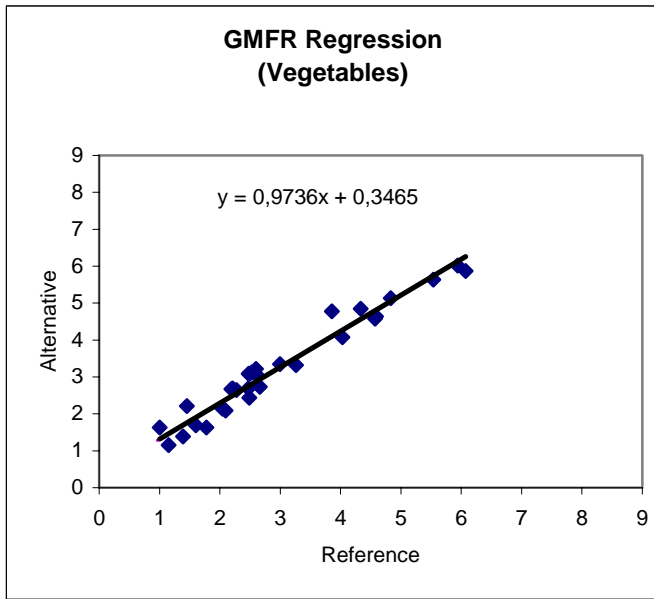
Annex 2 - Relative linearity: regression equation





Annex 3 - Relative accuracy: regression equation





All products

