ACCREDITATION
N°1-1836
SCOPE AVAILABLE ON
WWW.COFRAC.FR

PROFICIENCY TEST « RAEMA »

raema
réseau d'analyses et d'échanges en microbiologie des aliments

SCHEME N° 72
(8th MARCH 2021)
GENERAL REPORT

V. CARLIER[(1)], L. ALI-MANDJEE et M. CARLIER
ASA - ENVA, 7 avenue du Général de Gaulle, 94704 MAISONS ALFORT CEDEX

[(1)] Coordinator of the proficiency test « RAEMA »

For any claim, you can use the specific form available on our Website https://association.asa-spv.fr

Table of contents

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

1- GENERAL DATA

1.1. PARTICIPATING LABORATORIES

341 laboratories participated to the 72th scheme. The sending was made on Monday 8th March 2021. We received **337** answers (98.8%).

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+11	J0+14	J0+16
Nb of laboratories	8	208	54	23	21	4	2	8	3	1	1	1	1	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

The sample included :
- one strain of *Enterococcus sp*. at a concentration level of 10^5 cfu/g in 5 units ;
- one strain of *Citrobacter sp.* at a concentration level of 10^3 cfu/g in 5 units ;
- one strain of *Serratia liquefaciens* at a concentration level of 5.10^2 cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of 2.10^2 cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of 10^2 cfu/g in 2 units ;
- one strain of *Staphylococcus aureus* at a concentration level of 3.10^3 cfu/g in 5 units ;
- one strain of *Salmonella* Anatum at a concentration level of 25 cfu/g in 1 unit ;
- one strain of *Listeria monocytogenes* at a concentration level of 6.10^2 cfu/g in 3 units .

1.3.2. SIZE

180 kilogrammes of powder were produced, and distributed after contamination in bottles containing 75 grammes at least. Bottles were covered by a label with a 6 digit identification number.

1.3.3. HOMOGENEITY AND STABILITY TEST OF THE CONTAMINATION

Homogeneity and stability of samples are checked during the statistical analysis of participants results.
A supplementary check of the contamination's homogeneity was realized on 10 samples for each unit by a double enumeration of aerobic microorganisms at 30°C.
The contamination's stability was also checked by enumeration / detection of all flora on 15, 22 and 29 March 2021. These checks were realized by a subcontractor accreditated by Cofrac.
Homogeneity and stability of samples have been validated.

1.3.4. FLORA FOR ENUMERATION / DETECTION

Enumeration of the following flora was proposed : microorganisms at 30°C, Enterobacteriaceae, total and thermotolerant coliforms, beta-glucuronidase positive *Escherichia coli*, anaerobic sulfite-reducing bacteria, *Clostridium perfringens*, coagulase positive staphylococci, *Listeria monocytogenes*, as well as detection of *Salmonella* and *Listeria monocytogenes*.

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

1.4.EXECUTION OF ANALYZES

1.4.1. DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

336 laboratories (99.7%) specified it.

Analysis time	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+11	J0+14	J0+15	J0+18	J0+21
Nb of laboratories	0	40	48	34	5	1	1	141	46	6	2	1	6	3	1	1

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

334 laboratories (97.9%) specified it. The average temperature is **3.9°C** with a standard deviation of 0.8°C. The given data 14, 18, 20, 25 and 30°C given by 5 laboratories were not taken into account for this calculation.

2. EXPLOITATION OF ANALYSIS REPORT

2.1.PREPARATION OF THE INITIAL SUSPENSION

For **337** answers (100%) :
216 laboratories (64.1%) prepare the initial suspension with adding diluent to powder.
121 laboratories (35.9%) prepare the initial suspension with adding powder to diluent.

2.2.DILUENT USED FOR THE INITIAL SUSPENSION

For **335** answers (99.4%) :
270 laboratories (80.1%) use Buffered Peptone Water for the initial suspension.
56 laboratories (16.6%) use Peptone salt for the initial suspension.
9 laboratories (2.7%) used another diluent for the initial suspension.

2.3.HOMOGENEIZATION TECHNIQUE

For **336** answers (99.7%) :
313 laboratories (92.9%) homogeneize their sampling with a Stomacher[ND].
23 laboratories (6.8%) used another technique (manual, magnétic or other).

2.4.RESUSCITATION'S CONDITIONS

2.4.1. DURATION

315 laboratories (93.5%) specified it.
The average duration is **27.4 min** with a standard deviation of 14.8 min. The data 90, 120, 180 and 1440 min given by 6 laboratories was not taken into account for this calculation.

2.4.2. TEMPERATURE

322 laboratories (95.5%) specified it.
The average temperature is **21.3°C** with a standard deviation of 3.3°C.

2.5.MICROORGANISM AT 30°C

321 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 4833-1	203
	AFNOR 3M-01/1-09/89	48
	NM ISO 4833-1	26
	AFNOR BIO-12/35-05/13	13
	ISO/NF EN ISO 4833-2	10
	XP V08-034	5
	Other	16
	+ V08-100 (spiral)	16
Culture medium	Plate Count Agar	239
	Petrifilms	48
	Plate Count Agar + Milk	18
	Tempo AC	14
	Other	1
Preparation	Home made	115
	Ready to use not pre-poured	132
	Ready to use, plate, film, card	72
Plating method	Surface	64
	Pour	235
	Culture medium for card	15
1st dilution retained	- 1	13
	- 2	13
	- 3	267
	- 4	17
	- 5	1
	1/400	4
	1/4000	1
Incubation temperature	30°C	314
	23-25°C	3
	37°C	2
	33-35°C	2
Incubation duration	68-76 h	272
	44-48 h	47
	24-26 h	2

2.6.ENTEROBACTERIACEA

281 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-054	107
	→ *NM 08.0.109*[(1)]	22
	ISO/NF EN ISO 21528-2	73
	AFNOR 3M-01/6-09/97	46
	AFNOR BIO-12/21-12/06	9
	AFNOR AES-10/07-01/08	8
	AFNOR BRD-07/24-11/13	7
	Other	9
	+ V08-100 (spiral)	1
Culture medium	VRBG	205
	Petrifilms	48
	Tempo EB	10
	Rebecca	9
	Rapid'Enterobacteriaceae	8
	Other	1
Preparation	Home made	89
	Ready to use not pre-poured	129
	Ready to use, plate, film, card	62
1st dilution retained	- 1	209
	- 2	64
	- 3	1
	1/40	2
	1/400	5
Incubation temperature	37±1°C	176
	30°C	95
	35°C	10
Incubation duration	20-25 h	274
	48 h	7
Confirmatory test	Yes	66
	No	209

(1) *Similar method to NF V08-054 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

2.7.TOTAL COLIFORMS

233 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-050	118
	→ *NM 08.0.142*[(2)]	10
	ISO/NF ISO 4832	50
	AFNOR 3M	23
	NM ISO 4832	19
	AFNOR BIO-12/17-12/05	4
	AFNOR BRD-07/08-12/04	4
	Other	5
	+ V08-100 (spiral)	1
Culture medium	VRBL	196
	Petrifilms	25
	Rapid Ecoli	5
	Tempo TC	4
	Other	3
Preparation	Home made	88
	Ready to use not pre-poured	116
	Ready to use, plate, film, card	28
1st dilution retained	-1	208
	-2	22
	1/40	1
	1/400	2
Incubation temperature	30°C	218
	35°C	15
Incubation duration	18-25 h	229
	48 h	4

AFNOR 3M method including :

3 laboratories specified utilization of AFNOR 3M-01/02-09/89 A method.
1 laboratory specified utilization of AFNOR 3M-01/02-09/89 method.
1 laboratory specified utilization of AFNOR 3M Petrifilm CC method.
1 laboratory specified utilization of AFNOR 3M Petrifilm coliforme method.

(2) *Similar method to NF V 08-050 according to ONSSA.*

2.8.THERMOTOLERANT COLIFORMS

214 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-060	145
	→ *NM 08.0.124*(3)	26
	AFNOR 3M	25
	ISO/NF ISO 4832	12
	Other	5
	+ V08-100 (spiral)	2
Culture medium	VRBL	181
	Petrifilms	28
	Other	4
Preparation	Home made	84
	Ready to use not pre-poured	103
	Ready to use, plate, film, card	26
1st dilution retained	-1	195
	-2	18
Incubation temperature	42-48°C	209
	37°C	2
	30°C	2
Incubation duration	15-25 h	209
	48 h	4

AFNOR 3M method including :

3 laboratories specified utilization of AFNOR 3M-01/02-09/89 C method.
1 laboratory specified utilization of AFNOR 3M-01/02-09/89 method.
1 laboratory specified utilization of AFNOR 3M-01/05-03/97 method.
1 laboratory specified utilization of AFNOR 3M Petrifilm CC method.
1 laboratory specified utilization of AFNOR 3M Petrifilm coliforme method.
1 laboratory specified utilization of AFNOR 3M Petrifilm EC method.

(3) *Similar method to NF V08-060 according to ONSSA.*

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

2.9.ESCHERICHIA COLI

298 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 16649-2	168
	AFNOR 3M	45
	NM ISO 16649-2	23
	AFNOR BRD-07/01-07/93	17
	AFNOR BIO-12/13-02/05	11
	AFNOR AES-10/06-01/08	8
	NM 08.0.108	6
	AFNOR BIO-12/05-01/99	5
	NF EN ISO 16649-3	2
	Other	12
	+ V08-100 (spiral)	1
Culture medium	TBX	199
	Petrifilms	46
	Rapid E. coli	23
	Tempo EC	11
	Rebecca	10
	Coli ID	7
	Other	2
Preparation	Home made	89
	Ready to use not pre-poured	150
	Ready to use, plate, film, card	57
Plating method	Surface	41
	Pour	239
	Culture medium for card	13
1st dilution retained	-1	277
	-2	11
	1/40	2
	1/400	6
Incubation temperature	41-45°C	264
	37±1°C	33
	30°C	1
Incubation duration	18-26 h	291
	48 h	6
	34 h	1

AFNOR 3M method including :

14 laboratories specified utilization of AFNOR 3M-01/08-06/01 (*SELECT'E. COLI*) method.

1 laboratory specified utilization of AFNOR 3M-01/04-09/92 method.

1 laboratory specified utilization of AFNOR 3M Petrifilm EC method.

2.10. ANAEROBIC SULFITE-REDUCING BACTERIA

240 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-061	154
	→ *NM 08.0.154*[4]	4
	→ *NM 08.0.125*[4]	14
	ISO/NF ISO 15213	45
	NM ISO 15213	12
	Other	11
Culture medium	TSC	221
	Iron Sulfite agar	8
	TSN	8
	Other	3
Preparation	Home made	91
	Ready to use not pre-poured	122
	Ready to use, plate, film, card	27
Seeding way	Plates	150
	Tubes	89
1st dilution retained	-1	228
	-2	11
Incubation temperature	44-50°C	171
	37±1°C	67
	30°C	2
Incubation duration	16-24 h	201
	44-48 h	32
	72 h	7

[4] *Similar method to NF V08-061 according to ONSSA.*

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

2.11. CLOSTRIDIUM PERFRINGENS

196 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 7937	159
	NM ISO 7937	24
	NM 08.0.111	2
	Other	11
Culture medium	TSC	191
	Other	3
Preparation	Home made	64
	Ready to use not pre-poured	123
	Ready to use, plate, film, card	7
1st dilution retained	-1	190
	-2	4
Incubation temperature	37°C	185
	44-46°C	11
Incubation duration	18-24 h	192
	48 h	5
	72 h	1
Confirmation test	None	33
	Lactose-sulfite	136
	Strip	9
	Other	5

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

2.12. COAGULASE POSITIVE STAPHYLOCOCCI

300 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 6888-2	152
	ISO/NF EN ISO 6888-1	66
	NM ISO 6888-1	20
	AFNOR 3M-01/9-04/03	16
	AFNOR BKR-23/10-12/15	14
	AFNOR BIO-12/28-04/10	10
	NM ISO 6888-2	4
	NordVal No :049	2
	Other	14
	+ V08-100 (spiral)	5
Culture medium	RPF	144
	BP+egg yolk tellurite	86
	BP+egg yolk tellurite+ sulfamethazine	18
	Easy Staph	18
	Petrifilm	17
	Tempo STA	10
	Rapid Staph	3
	Other	3
Preparation	Home made	72
	Ready to use not pre-poured	124
	Ready to use, plate, film, cards	100
Plating method	Surface	148
	Pour	136
	Culture medium for card	10
1st dilution retained	-1	112
	-2	176
	-3	4
	1/40	3
	1/400	4
Incubation temperature	37±1°C	295
	27-30°C	3
	44°C	1
Incubation duration	42-48 h	208
	18-26 h	88
	72 h	1
	36-39 h	2
Confirmation test	None	187
	Staphylo-coagulase	82
	Clumping factor	7
	DNase	11
	Other	4

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

2.13. LISTERIA MONOCYTOGENES – ENUMERATION

241 laboratories performed the enumeration.

RESUSCITATION

64 laboratories announce the realization of a resuscitation step.
The average duration for these laboratories is **45.0 min** with a standard deviation of 18.7 min.
The average temperature for these laboratories is **21.3°C** with a standard deviation of 3.1°C.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-2	69
	AFNOR AES-10/05-09/06	61
	AFNOR BKR-23/05-12/07	51
	AFNOR BRD-07/05-09/01	22
	NM ISO 11290-2	22
	AFNOR BRD-07/17-01/09	8
	Other	6
Resuscitation step	Yes	70
	No	158
Resuscitation medium	Buffered Peptone Water	54
	Fraser base	13
	Other	2
Enumeration medium	ALOA Count	117
	Compass Listeria	77
	Rapid Lmono	23
	AL Agar	14
	OCLA	3
	Palcam	3
	Other	3
Preparation	Home made	31
	Ready to use not pre-poured	54
	Ready to use, plate, film, card	153
Plating method	Surface	195
	Pour	42
	Culture medium for card	0

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

Parameters	Mode	Nb laboratories
1st dilution retained	-1	226
	-2	14
Incubation temperature	37°C	237
	30°C	2
Incubation duration	43-49 h	195
	24 h	44
Confirmation test	None	47
	Biochemical	138
	Biochemical + CAMP	37
	Other	10
Nb of colonies tested per plate	1	57
	2-4	20
	5	98
	6	1

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

2.14. SALMONELLA – DETECTION

306 laboratories performed the detection.
Methods used by laboratories are clarified in the following table :

Parameter	Mode	Nb laboratories
Method	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	74
	ISO/NF EN ISO 6579-1	73
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	33
	NM ISO 6579-1	33
	AFNOR BIO 12/41-03/17 (SALMA One day)	25
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	24
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	23
	Other	21

No detail of methodology was asked to laboratories using other methods than ISO/NF EN ISO 6579-1 and NM ISO 6579-1 methods and proposed in the questionnaire.

You will find below a short description of these methods :

Method	Pre-enrichment	Enrichment	Isolation
AFNOR BIO 12/16-09/05 **VIDAS Easy Salmonella**	BPW / 37°C - 16/20h	SX2 / 41,5°C - 22/26h	Chrom ID / 37°C - 24h
AFNOR BIO 12/32-10/11 **VIDAS SPT**		BPW + Salmonella supplement / 41,5°C - 18/24h	Chrom ID / 37°C - 24h
AFNOR BKR 23/07-10/11 **IRIS Salmonella**		IRIS Salmonella Enrichment / 41,5°C - 18±2h	IRIS / 37°C - 24±3h
AFNOR BRD 07/11-12/05 **Rapid Salmonella**		BPW + Salmonella capsule / 41,5°C - 18±2h	Rapid Salmonella / 37°C - 24±2h
AFNOR BIO 12/41-03/17 **SALMA One day**		BPW + Salmonella supplement / 41.5°C – 16/24h	SALMA / 37°C - 24±3h

The detail of the methodology followed by 106 laboratories using ISO/NF EN ISO 6579-1 and NM ISO 6579-1 method, and the 21 laboratories using another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 6579-1	73
	NM ISO 6579-1	33
	Other	21
Pre-enrichment medium	Buffered Peptone Water	118
	Other	8
Pre-enrichment temperature	37±1°C	117
	41-42.5°C	7
	20-22°C	2
Pre-enrichment duration	16-20 h	91
	21-24 h	34
	8 h	1
Enrichment medium	RVS	108
	MKTTn	101
	Selenite-cystine broth	24
	Other	5
Isolation medium	XLD	100
	Hektoen	33
	Bismuth Sulfate	22
	ASAP	13
	IRIS Salmonella agar	10
	Brilliance Salmonella	10
	GVB	10
	SS	8
	Rapid Salmonella	7
	Compass Salmonella	6
	Rambach	3
	Other	11
Confirmation test	Biochemical	55
	Biochemical + serological agglutination	61
	Other	5

2.15. LISTERIA MONOCYTOGENES – DETECTION

272 laboratories performed the detection.

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	64
	AFNOR AES 10/03-09/00 (ALOA one day)	58
	AFNOR BKR 23/02-11/02 (Compass L. mono)	54
	NM ISO 11290-1	26
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	23
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	9
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	7
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	6
	AFNOR BRD 07/16-01/09 (Agar Listeria)	5
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	4
	AFNOR UNI 03/04-04/05 (Listeria Precis)	3
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	2
	Other	11

No detail of methodology was asked to laboratories using other method than ISO/NF EN ISO 11290-1 and NM ISO 11290-1 methods and proposed in the questionnaire.

You will find below a short description of these methods :

Méthod	Primary enrichment		Secondary enrichment		Isolation
	Medium	Incubation	Medium	Incubation	
AFNOR BRD 07/04-09/98 **Rapid' L. mono**	Fraser 1/2	30°C - 24±2h			Rapid L'mono 37°C – 24h
AFNOR BIO 12/02-06/94 **VIDAS Listeria**	Fraser 1/2	37°C - 26/30h	Fraser	30°C - 24/26h	Palcam et Oxford 37°C – 24h
AFNOR BIO 12/27-02/10 **VIDAS LMX**	LMX	37°C - 26/30h			ChromID 37°C – 24h
AFNOR BIO 12/11-03/04 **VIDAS LMO2 (37°C)**	Fraser 1/2	30°C - 24/26h	Fraser	37°C - 24/26h	Chromogenic medium / Palcam / Oxford
AFNOR AES 10/03-09/00 **ALOA one day**	Fraser 1/2	30°C - 24±2h			ALOA One Day 37°C – 24/48h
AFNOR BKR 23/02-11/02 **Compass L. mono**	Fraser 1/2	30°C - 24±2h			Compass Listeria Agar 37°C – 24h
AFNOR BRD 07/16-01/09 **Agar Listeria**	Fraser 1/2	30°C - 24±2h			Agar Listeria 37°C – 24h
AFNOR UNI 03/04-04/05 **Listeria Precis**	One Broth Listeria	30°C - 24±2h			Brilliance Listeria 37°C – 24h
AFNOR BIO 12/40-11/16 **GENE UP LMO**	LPT	35-37°C - 24±2h			ALOA 35-37°C – 24/48h
AFNOR BIO 12/18-03/06 **VIDAS LDUO**	LX	30°C - 24±2h	LX	30°C - 24/26h	Chromogenic medium / Palcam / Oxford

The detail of the methodology followed by 90 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 methods, and the 11 laboratories using another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	64
	NM ISO 11290-1	26
	Other	11
Primary enrichment medium	Half-Fraser	87
	One broth Listeria	3
	Other	11
Primary enrichment temperature	30°C	92
	37°C	7
	25°C	1
Primary enrichment duration	22-28 h	95
	48 h	2
	1 h	1
Secondary enrichment medium	Fraser	87
	Other	1
Secondary enrichment temperature	37°C	83
	30°C	3
	25°C	1
Secondary enrichment duration	23-27 h	63
	45-48 h	22
	1 h	1
Isolation medium	Palcam	69
	Ottaviani et Agosti	54
	Compass Listeria	31
	Oxford	14
	Rapid L'mono	5
	Brilliance Listeria	3
	Other	3
Isolation temperature	37°C	94
	30°C	3
Isolation duration	48 h	63
	24 h	34
Confirmation test	None	6
	Biochemical	56
	Biochemical + CAMP	30
	Other	4
Nb of colonies per plate	1	26
	2-4	11
	5	46

3. ASSESSMENT OF PERFORMANCE (INDIVIDUEL REPORTS)

3.1.PERFORMANCES IN ENUMERATION

Performance is assessed on two criteria : **precision and trueness**.
The assigned value of the contamination used to assess the trueness and the reference standard deviation for the assessment of the precision are consensual values obtained with the results of all the participants. These values are obtained by robust estimation methods in order to eliminate influence of aberrant results. However some results are excluded of the statistical analysis. That is the case when laboratories do not give results for all contaminated units, when results are “less than CFU/g“, when samples are analyzed after the deadline (time of receipt > 4 days after sending or time of analysis >15 days after sending) or when this information is not specified.

A statistical analysis has also be done to highlight potential relations between techniques used (delay of analysis, preservation temperature, preparation of the initial suspension, homogenization technique, resuscitation conditions , method used, media used, manufacturers of media, preparation mode, plating method, incubation conditions) and results obtained. We need to clarify that this statistical link is not involved in a cause - effect relationship. Indeed, this link may be due to a not documented factor.

When a significant statistical link is identified between use of a technique and the obtained results, the assessment of performance is done considering the influence of one or several factors involved if their effect translates into a contamination’s difference higher than 0.15 log CFU/g for non-selective media or higher than 0.30 log CFU/g for selective media (these limits match with productivity limits of culture media usually recommended in the standard NF EN ISO 11133).

PRECISION
The precision reflects the repeatability (or reproducibility intra-laboratory) of your work.

The standard deviation of your results, *s*, is compared to the robust estimation of the standard deviation (reference standard deviation of precision), *s**, obtained with algorithm S from the standard ISO 13528 applied to all standard deviations obtained by laboratories included in the statistical analysis.

An index score is then calculated using the following formula : $i = (k-1) \cdot \frac{s^2}{s^{*2}}$ (with k, number of contaminated units and retained in the statistical analysis, usually 5).

The standard ISO 13528 do not provide warning and action limits for this score, so its interpretation is left to your discretion.

As an indicator, we suggest following values by analogy with those indicated for the evaluation of trueness.
For k=5, a score lower than 0.1 or higher than 18 may be considered as an action signal and a score lower than 0.45 or higher than 11.5 may be considered as a warning signal.
For k=4, a score lower than 0.03 or higher than 15.5 may be considered as an action signal and a score lower than 0.2 or higher than 9.5 may be considered as a warning signal.
For k=3, a score lower than 0.003 or higher than 13.2 may be considered as an action signal and a score lower than 0.05 or higher than 7.5 may be considered as a warning signal.
For k=2, a score lower than 0.000002 or higher than 10.3 may be considered as an action signal and a score lower than 0.0008 or higher than 5.2 may be considered as a warning signal.

TRUENESS

The trueness reflects the closeness of the mean of your results to the contamination's assigned value of samples. It has been evaluated for all enumerated flora.

The mean of your results in log CFU/g, *m* (on contaminated units and included in the statistical analysis), is compared to the contamination's assigned value, m_{pt}, obtained with algorithm A from the standard ISO 13528 applied to all laboratories mean included in the statistical analysis. When groups are formed, each one is characterize by its own assigned value.

A z score is then calculated with the following formula : $z = \frac{m - m_{pt}}{\sigma_{pt}}$, where σ_{pt} is the standard deviation for proficiency assessment (robust estimation of the standard deviation obtained by participants).

The standard ISO 13528 specifies that *z* score lower than -3 or higher than +3 must be considered as an action signal and that a z score lower than -2 or higher than +2 must be considered as a warning signal.

In this report, we specify, estimations of interlaboratories standard deviation for enumerations proposed as well as reproducibility standard deviation or global standard deviation for the test (parameters including interlaboratories variability and the variability of the precision).

INDIVIDUAL REPORTS – FOR EACH CRITERIA YOU FIND THE FOLLOWING INFORMATIONS

- your results in logarithm base 10 (-1 when the answer is < limit and NaN when there is no answer). Comment : the presentation order of your results does not necessarily correspond to the order you sent them, this order is the same for all the flora.
- histogram for the studied parameter (laboratories standard deviations for the precision and laboratorie's means for the trueness) with an asterisk indicating the location of your result,
- standard deviation (precision) or mean (trueness) of your results (on contaminated units and retained in the statistical analysis),
- the method declared in your results input,
- when necessary, your group in relation to the technique used,
- precision score or z score,
- number of laboratories which made analysis (and belonging to your group),
- number of laboratories included in the statistical analysis,
- reference standard deviation for the precision or assigned value of the contamination and standard deviation aptitude assessment (trueness),
- number of laboratories with a satisfactory signal,
- number of laboratories with a warning signal,
- number of laboratories with an action signal.

3.1.1. MICROORGANISMS AT 30°C

None significant effect of the analysis technique has been highlighted.

Microorganisms at 30°C	
Assigned value of the contamination (log CFU/g)	5.171
Assigned value uncertainty (log CFU/g)	0.0061
Standard deviation for proficiency assessment (log CFU/g)	0.0844
Standard deviation for precision (log CFU/g)	0.0528
Interlaboratory's standard deviation (log CFU/g)	0.0810
Reproducibility standard deviation (log CFU/g)	0.0967

3.1.2. ENTEROBACTERIACEAE

A significant "effect" of the preparation mode of the initial suspension, culture media, manufacturer and the retained dilution has been highlighted. This effect results in a contamination's difference higher than 0.3 log CFU/g, then results have been gathered in three groups :

Enterobacteriaceae	Group 1	Group 2	Group 3
Assigned value of the contamination (log CFU/g)	2.707	3.039	3.221
Assigned value uncertainty (log CFU/g)	0.0217	0.0882	0.0229
Standard deviation for proficiency assessment (log CFU/g)	0.2452	0.2992	0.1203
Standard deviation for precision (log CFU/g)		0.0980	
Interlaboratory's standard deviation (log CFU/g)	0.2413	0.2960	0.1120
Reproducibility standard deviation (log CFU/g)	0.2604	0.3118	0.1488

3.1.3. TOTAL COLIFORMS

A significant "effect" of the culture media, manufacturer, preparation mode and the retained dilution has been highlighted. This effect results in a contamination's difference higher than 0.3 log CFU/g, then results have been gathered in three groups :

Total coliforms	Group 1	Group 2	Group 3
Assigned value of the contamination (log CFU/g)	2.463	2.701	3.096
Assigned value uncertainty (log CFU/g)	0.0315	0.0278	0.0548
Standard deviation for proficiency assessment (log CFU/g)	0.2139	0.2385	0.2319
Standard deviation for precision (log CFU/g)		0.0989	
Interlaboratory's standard deviation (log CFU/g)	0.2093	0.2344	0.2276
Reproducibility standard deviation (log CFU/g)	0.2311	0.2540	0.2478

Association ASA – Bât. Jean GIRARD, Ecole Nationale Vétérinaire d'Alfort, 7 av du Général de Gaulle, 94704 Maisons Alfort Cedex

3.1.4. THERMOTOLERANT COLIFORMS

A significant “effect” of the culture media manufacturer has been highlighted. This effect results in a contamination’s difference lower than 0.3 log CFU/g, then results have been gathered in one group :

Thermotolerant coliforms	
Assigned value of the contamination (log CFU/g)	2.606
Assigned value uncertainty (log CFU/g)	0.0232
Standard deviation for proficiency assessment (log CFU/g)	0.2600
Standard deviation for precision (log CFU/g)	0.1116
Interlaboratory’s standard deviation (log CFU/g)	0.2552
Reproducibility standard deviation (log CFU/g)	0.2785

3.1.5. *ESCHERICHIA COLI*

A significant “effect” of the preparation mode of the initial suspension and the plating method has been highlighted. This effect results in a contamination’s difference lower than 0.3 log CFU/g, then results have been gathered in one group :

Escherichia coli	
Assigned value of the contamination (log CFU/g)	2.430
Assigned value uncertainty (log CFU/g)	0.0156
Standard deviation for proficiency assessment (log CFU/g)	0.2071
Standard deviation for precision (log CFU/g)	0.1148
Interlaboratory’s standard deviation (log CFU/g)	0.2007
Reproducibility standard deviation (log CFU/g)	0.2312

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°3 and 5 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

Anaerobic sulfite-reducing bacteria	
Assigned value of the contamination (log CFU/g)	2.228
Assigned value uncertainty (log CFU/g)	0.0168
Standard deviation for proficiency assessment (log CFU/g)	0.1972
Standard deviation for precision (log CFU/g)	0.1280
Interlaboratory’s standard deviation (log CFU/g)	0.1752
Reproducibility standard deviation (log CFU/g)	0.2170

Comment :
- 6 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 130 to 2500 CFU/g.
- 9 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 1 to 2600 CFU/g.
- 10 laboratories detected ASR in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 171 to 2300 CFU/g.

3.1.7. *CLOSTRIDIUM PERFRINGENS*

Only units n°3 and 5 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

Clostridium perfringens	
Assigned value of the contamination (log CFU/g)	2.243
Assigned value uncertainty (log CFU/g)	0.0168
Standard deviation for proficiency assessment (log CFU/g)	0.1807
Standard deviation for precision (log CFU/g)	0.0980
Interlaboratory's standard deviation (log CFU/g)	0.1669
Reproducibility standard deviation (log CFU/g)	0.1936

Comment :
- 3 laboratories detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 140 to 2000 CFU/g.
- 2 laboratories detected *C. perfringens* in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 63 to 2100 CFU/g.
- 5 laboratories detected *C. perfringens* in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 10 to 1800 CFU/g.

3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

A significant "effect" of the resuscitation's duration has been highlighted. This effect results in a contamination's difference lower than 0.3 log CFU/g, then results have been gathered in one group :

Coagulase positive Staphylococci	
Assigned value of the contamination (log CFU/g)	3.583
Assigned value uncertainty (log CFU/g)	0.0116
Standard deviation for proficiency assessment (log CFU/g)	0.1549
Standard deviation for precision (log CFU/g)	0.0727
Interlaboratory's standard deviation (log CFU/g)	0.1515
Reproducibility standard deviation (log CFU/g)	0.1680

3.1.9. *LISTERIA MONOCYTOGENES*

Only units n°3, 4 and 5 were artificially contaminated.

A significant "effect" of the preparation mode of the initial suspension has been highlighted. This effect results in a contamination's difference lower than 0.3 log CFU/g, then results have been gathered in one group :

Listeria monocytogenes	
Assigned value of the contamination (log CFU/g)	2.789
Assigned value uncertainty (log CFU/g)	0.0089
Standard deviation for proficiency assessment (log CFU/g)	0.1066
Standard deviation for precision (log CFU/g)	0.0726
Interlaboratory's standard deviation (log CFU/g)	0.0980
Reproducibility standard deviation (log CFU/g)	0.1220

3.2.PERFORMANCES IN DETECTION

The performance is assessed by the capacity to detect only samples contaminated by *Salmonella* and *Listeria monocytogenes* (no false positive or false negative results).

3.2.1. DETECTION – *SALMONELLA*

Only unit n°3 was artificially contaminated.
300 laboratories obtained correct results.
4 laboratories obtained false positive results (respectively 3, 2, 4 and 1 false-positive for units n°1, 2, 4 and 5).
2 laboratories obtained false negative results for unit n°3.

3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

Only units n°3, 4 and 5 were artificially contaminated.
261 laboratories obtained correct results.
2 laboratories obtained false positive results (respectively 2 and 1 false-positive for units n°1 and 2).
5 laboratories obtained false negative results (respectively 2, 4 and 2 false-negative for units n°3, 4 and 5).

3.3.EVOLUTION OF PERFORMANCE

You will find, on each page of your performance's assessment, a graph representing evolution of it on different tests since the 52th scheme.

In order to interpret your control card with z scores, you can refer to the standard ISO 13528 §10.8.2.2, explaining the 3 « out of control » situations :

- Just one overtaking of the action limit (z<-3 or z>3),
- 2 consecutives z scores out of 3 overtaking of the warning limit (2<z<3 or -3<z<-2),
- 6 consecutives z scores regularly increasing or decreasing.