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PROFICIENCY TEST « RAEMA »

POWDER SCHEME N° 78
(12th MARCH 2024)
GENERAL REPORT

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Table of contents

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1- GENERAL DATA

1.1. PARTICIPATING LABORATORIES

338 laboratories participated to the 78th scheme. The sending was made on Tuesday 12th March 2024. We received **335** answers (99.1%).

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+6	J0+7	J0+8	J0+9	J0+10	J0+14
Nb of laboratories	22	219	42	23	13	9	2	3	1	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

The sample included :

- one strain of *Enterococcus sp*. at a concentration level of 2.10^5 cfu/g in 5 units ;
- one strain of *Citrobacter sp.* at a concentration level of 5.10^2 cfu/g in 5 units ;
- one strain of *Serratia liquefaciens* at a concentration level of 4.10^2 cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of 3.10^2 cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of 1.10^3 cfu/g in 4 units ;
- one strain of *Staphylococcus aureus* at a concentration level of 6.10^3 cfu/g in 5 units ;
- one strain of *Salmonella* Anatum at a concentration level of 500 cfu/g in 2 units ;
- one strain of *Listeria monocytogenes* at a concentration level of 5.10^3 cfu/g in 2 units.

Samples have been prepared between January and March 2024. The maintenance of bacterial strains and check of their contamination are entrusted to a subcontractor.

1.3.2. SIZE

180 kilogrammes of milk 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 18, 25 March and 2nd April 2024. These checks were realized by a subcontractor accreditated by Cofrac.
Homogeneity has been validated, except for "Enterobacteriaceae" and "Thermotolerant coliforms" parameters. For those two parameters, homogeneity between units has not been validated, then, units 1 and 3 were not retained in the statistical analysis. Only units 2, 4 and 5 were considered for the assessment of trueness and precision.
Stability has been validated, except for the "Thermotolerant coliforms" parameter in the 3rd week of analysis. Only laboratories which having analyzed this parameter from J13 are concerned and informed.

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*.

1.4. EXECUTION OF ANALYZES

1.4.1. DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

335 laboratories (100%) specified it.

Analysis time	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+11	J0+12	J0+13	J0+14	J0+15	J0+16	J0+21
Nb of laboratories	2	42	36	11	2	1	151	56	11	8	1	1	6	3	2	1	1

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

334 laboratories (99.7%) specified it. The average temperature is **3.9°C** with a standard deviation of 0.9°C. The given data -4, 20, 22 and 25°C given by 6 laboratories were not taken into account for this calculation.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF THE SAMPLES

331 laboratories specified it (98.8%).
The average size is **18.1 g** with a standard deviation of 8.0 g. The minimum size indicated is 1 g and the maximum one is 50 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

For **333** answers (99.4%) :
208 laboratories (62.1%) prepare the initial suspension with adding diluent to powder.
120 laboratories (35.8%) prepare the initial suspension with adding powder to diluent.
5 laboratories (1.5%) used another technique to prepare the initial suspension.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

For **330** answers (98.5%) :
291 laboratories (86.9%) use Buffered Peptone Water (or equivalent) for the initial suspension.
35 laboratories (10.5%) use Peptone salt for the initial suspension.
4 laboratories (1.1%) used another diluent for the initial suspension.

2.4. HOMOGENEIZATION TECHNIQUE

For **332** answers (99.1%) :
306 laboratories (91.3%) homogeneize their sampling with a StomacherND.
18 laboratories (5.4%) used a manual homogenization.
5 laboratories (1.5%) used a Vortex mixer.
3 laboratories (0.9%) used another technique.

2.5. RESUSCITATION'S CONDITIONS

2.5.1. DURATION

319 laboratories (95.2%) specified it.
The average duration is **27.0 min** with a standard deviation of 15.9 min. The data 1440 min given by one laboratory was not taken into account for this calculation.

2.5.2. TEMPERATURE

319 laboratories (95.2%) specified it.
The average temperature is **21.5°C** with a standard deviation of 3.3°C.

2.6. MICROORGANISM AT 30°C

318 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 4833-1	205
	AFNOR 3M-01/1-09/89	44
	NM ISO 4833-1	27
	ISO/NF EN ISO 4833-2	12
	AFNOR BIO-12/35-05/13	11
	Internal method	6
	XP V08-034	6
	Other	7
	+ Spiral metho	16
Culture medium	Plate Count Agar	244
	Petrifilms	45
	Plate Count Agar + Milk	17
	Tempo AC	11
	Other	1
Preparation	Home made	107
	Ready to use not pre-poured	141
	Ready to use, plate, film, card	70
Plating method	Surface	67
	Pour	235
	Culture medium for card	11
1st dilution retained	- 1	12
	- 2	13
	- 3	239
	- 4	44
	1/400	7
	1/4000	2
Incubation temperature	30°C	316
	33°C	1
	37°C	1
Incubation duration	69-75 h	272
	40-48 h	44
	24-26 h	2

2.7. ENTEROBACTERIACEAE

284 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-054	103
	→ *NM 08.0.109*(1)	17
	ISO/NF EN ISO 21528-2	78
	AFNOR 3M-01/6-09/97	46
	NM ISO 21528-2	13
	AFNOR AES-10/07-01/08	8
	AFNOR BRD-07/24-11/13	8
	AFNOR BIO-12/21-12/06	8
	Internal method	2
	Other	1
Culture medium	VRBG	210
	Petrifilms	48
	Rebecca	9
	Rapid'Enterobacteriaceae	8
	Tempo EB	8
	Other	1
Preparation	Home made	90
	Ready to use not pre-poured	134
	Ready to use, plate, film, card	60
1st dilution retained	- 1	228
	- 2	48
	1/40	2
	1/400	5
Incubation temperature	37°C	185
	30°C	89
	35°C	9
Incubation duration	22-25 h	278
	48 h	5
Confirmatory test	Yes	77
	No	196

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

2.8. TOTAL COLIFORMS

221 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-050	106
	→ *NM 08.0.142*(2)	7
	ISO/NF ISO 4832	60
	NM ISO 4832	24
	AFNOR 3M	11
	AFNOR BIO-12/17-12/05	4
	AFNOR BRD-07/08-12/04	4
	Other	5
Culture medium	VRBL	197
	Petrifilms	12
	Rapid Ecoli	5
	Tempo TC	4
	Other	3
Preparation	Home made	82
	Ready to use not pre-poured	122
	Ready to use, plate, film, card	17
1st dilution retained	-1	206
	-2	11
	1/400	3
Incubation temperature	30°C	207
	37°C	12
	24°C	1
Incubation duration	21-24 h	216
	48 h	4

AFNOR 3M method including :
1 laboratory specified utilization of AFNOR 3M-Petrifilm CC method.

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

2.9. THERMOTOLERANT COLIFORMS

197 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-060	137
	→ *NM 08.0.124*(3)	29
	AFNOR 3M	16
	ISO/NF ISO 4832	12
	Other	3
Culture medium	VRBL	177
	Petrifilms	16
	Other	4
Preparation	Home made	78
	Ready to use not pre-poured	104
	Ready to use, plate, film, card	15
1st dilution retained	-1	187
	-2	9
Incubation temperature	42-44.5°C	192
	37°C	3
	30°C	2
Incubation duration	22-24 h	193
	44-48 h	4

AFNOR 3M method including :

1 laboratory specified utilization of AFNOR 3M-01/02-09/89 C method.
1 laboratory specified utilization of AFNOR 3M-01/05-03/97 B method.
1 laboratory specified utilization of AFNOR 3M-Petrifilm EC method.
1 laboratory specified utilization of AFNOR 3M-Petrifilm high sensitivity method.

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

2.10. ESCHERICHIA COLI

296 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 16649-2	175
	AFNOR 3M	43
	NM ISO 16649-2	27
	AFNOR BRD-07/01-07/93	15
	AFNOR BIO-12/13-02/05	11
	AFNOR AES-10/06-01/08	6
	NM 08.0.108	4
	ISO/NF EN ISO 16649-3	4
	AFNOR BIO-12/05-01/99	3
	Internal method	3
	Other	5
Culture medium	TBX	208
	Petrifilms	44
	Rapid E. coli	18
	Tempo EC	11
	Rebecca	8
	Coli ID	5
	Other	1
Preparation	Home made	90
	Ready to use not pre-poured	151
	Ready to use, plate, film, card	53
Plating method	Surface	44
	Pour	235
	Culture medium for card	13
1st dilution retained	-1	276
	-2	10
	1/40	3
	1/400	6
Incubation temperature	40-46°C	268
	37°C	26
	30°C	1
Incubation duration	18-25 h	291
	48 h	4

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-Petrifilm EC method.
1 laboratory specified utilization of AFNOR 3M-01/01-09/89 method.

2.11. ANAEROBIC SULFITE-REDUCING BACTERIA

237 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-061	153
	→ *NM 08.0.125*(4)	16
	ISO/NF ISO 15213-1	41
	NM ISO 15213	16
	Internal method	5
	Other	5
Culture medium	TSC	211
	Iron Sulfite agar	18
	TSN	6
	Other	2
Preparation	Home made	83
	Ready to use not pre-poured	132
	Ready to use, plate, film, card	22
Seeding way	Plates	168
	Tubes	68
1st dilution retained	-1	130
	-2	100
	-3	6
Incubation temperature	44-46°C	169
	37°C	66
	30°C	1
Incubation duration	16-24 h	188
	48 ±1 h	44
	72 h	4

(4) *Similar method to NF V08-061 according to ONSSA.*

2.12. CLOSTRIDIUM PERFRINGENS

197 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 7937	125
	ISO/NF ISO 15213-2	31
	NM ISO 7937	29
	NM 08.0.111	3
	Internal method	1
	Other	8
Culture medium	TSC	197
Preparation	Home made	63
	Ready to use not pre-poured	130
	Ready to use, plate, film, card	4
1st dilution retained	-1	130
	-2	67
Incubation temperature	36.5-37°C	192
	44-46°C	5
Incubation duration	17-24 h	193
	48 h	4
Confirmation test	None	36
	Lactose-sulfite	128
	Strip	9
	SIM agar	7
	Acid phosphatase	4
	MALDI-TOF mass spectrometry	4
	Other	3

The standard ISO/NF ISO 15213-2 has been published in November 2023, it amends the standard ISO/NF EN ISO 7937. In this new standard, confirmation's methods have been revised ; requirements and features of media's performance have been especially added.

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2.13. COAGULASE POSITIVE STAPHYLOCOCCI

291 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 6888-2/A1	127
	ISO/NF EN ISO 6888-1/A1	74
	AFNOR BKR-23/10-12/15	27
	NM ISO 6888-1	24
	AFNOR 3M-01/9-04/03	13
	AFNOR BIO-12/28-04/10	8
	NM ISO 6888-2	3
	NM 08.0.112	3
	ISO/NF EN ISO 6888-3	2
	NordVal No :049	2
	Internal method	1
	Other	7
Culture medium	RPF	129
	BP+egg yolk tellurite	92
	Easy Staph	29
	Petrifilm	13
	BP+egg yolk tellurite+ sulfamethazine	13
	Tempo STA	8
	Rapid Staph	2
	Other	5
Preparation	Home made	76
	Ready to use not pre-poured	124
	Ready to use, plate, film, cards	91
Plating method	Surface	150
	Pour	130
	Culture medium for card	8
1st dilution retained	-1	109
	-2	172
	-3	2
	1/40	4
	1/400	3
Incubation temperature	35-37°C	289
	30°C	1
Incubation duration	42-49 h	207
	20-25 h	82
	32 h	1
Confirmation test	None	181
	Staphylo-coagulase	78
	Clumping factor	19
	DNase	7
	MALDI-TOF mass spectrometry	4
	Other	1

2.14. LISTERIA MONOCYTOGENES – ENUMERATION

243 laboratories performed the enumeration.

RESUSCITATION

73 laboratories announce the realization of a resuscitation step.

Details concerning temperature and average duration of this step are not required anymore in the input form.

Parameters	Mode	Nb laboratories
Method	AFNOR AES-10/05-09/06	63
	ISO/NF EN ISO 11290-2	62
	AFNOR BKR-23/05-12/07	57
	NM ISO 11290-2	27
	AFNOR BRD-07/05-09/01	25
	AFNOR BRD-07/17-01/09	8
	Other	1
Resuscitation medium	Buffered Peptone Water or equivalent	204
	Half-fraser	32
	Fraser base	4
Enumeration medium	ALOA Count	114
	Compass Listeria	88
	Rapid Lmono	25
	AL Agar	11
	OCLA	3
	Palcam	2
Preparation	Home made	41
	Ready to use not pre-poured	49
	Ready to use, plate, film, card	152
Plating method	Surface	200
	Pour	39

Parameters	Mode	Nb laboratories
1st dilution retained	-1	133
	-2	107
	-3	1
	1/2	1
Incubation temperature	37±1°C	241
	30°C	2
Incubation duration	42-48.5 h	200
	21-24 h	43
Confirmation test	None	47
	Biochemical	144
	Biochemical + CAMP	33
	MALDI-TOF mass spectrometry	6
	Other	3
Nb of colonies tested per plate	1	58
	2-3	14
	5	106
	10	1
	120	1
	150	1

2.15. SALMONELLA – DETECTION

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

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 6579-1	81
	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	73
	NM ISO 6579-1	39
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	36
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	21
	AFNOR BIO 12/41-03/17 (SALMA One day)	18
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	11
	AFNOR BIO 12/01-04/94 (VIDAS SLM)	8
	AFNOR UNI 03/06-12/07 (Salmonella precis)	4
	AFNOR UNI 03/07-11/13 (PCR)	3
	AFNOR BIO 12/38-06/16 (GENE UP Salmonella)	3
	AFNOR BRD 07/06-07/04 (PCR)	2
	AFNOR TRA 02/08-03/01 (TRANSIA PLATE Salmonelle GOLD)	1
	Other	5

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 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 12/32-10/11 **VIDAS SPT**		BPW + Salmonella supplement / 41,5°C - 18/24h	Chrom ID / 37°C - 24h
AFNOR BIO 12/41-03/17 **SALMA One day**		BPW + Salmonella supplement / 41.5°C – 16/24h	SALMA / 37°C - 24±3h
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 12/01-04/94 **VIDAS SLM**	BPW / 35°C – 24±2h	Tetrathionate (42°C - 6/8h) – Selenite cystine (35-37°C – 6/8h) + M-Broth (42°C – 18h)	Vidas Heat & Go
AFNOR UNI 03/07-11/13 **PCR**		BPW + supplement / 34-38°C – 20/24h	Lysis + PCR
AFNOR UNI 03/06-12/07 **Salmonella precis**		One broth-Salmonella / 42°C – 16/24h	Brilliance Salmonella / 37°C – 24±2h
AFNOR BIO 12/38-06/16 **GENE UP Salmonella**		BPW / 42°C – 18/24h	Lysis + PCR
AFNOR BRD 07/06-07/04 **PCR**		BPW / 37°C – 18/21h	Lysis + PCR
AFNOR TRA 02/08-03/01 **TRANSIA PLATE Salmonella GOLD**	BPW / 37°C – 16/20h	RVS / 41.5°C – 18/24h	ELISA test
AFNOR QUA 18/03-11/02 **BAX SYSTEM PCR**		BPW / 37°C – 16/20h	Lysis + PCR

The detail of the methodology followed by 120 laboratories using ISO/NF EN ISO 6579-1 and NM ISO 6579-1 methods, and the 5 laboratories using an internal or another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 6579-1	81
	NM ISO 6579-1	39
	Other	5
Pre-enrichment medium	None pre-enrichment	1
	Buffered Peptone Water	121
	Other	2
Pre-enrichment temperature	37±1°C	119
	41.5-42°C	3
	22°C	1
Pre-enrichment duration	16-20 h	88
	21-24 h	35
Enrichment medium	None enrichment	2
	RVS	116
	MKTTn	116
	Selenite-cystine broth	28
	Other	4
Isolation medium	XLD	114
	Hektoen	34
	Bismuth Sulfate	28
	IRIS Salmonella agar	16
	GVB	15
	ASAP	13
	SS	13
	Compass Salmonella	6
	Rapid Salmonella	5
	Rambach	1
	Brilliance Salmonella	1
	Other	11
Confirmation test	Biochemical	52
	Biochemical + serological agglutination	62
	MALDI-TOF mass spectrometry	6
	Other	2

2.16. LISTERIA MONOCYTOGENES – DETECTION

281 laboratories performed the detection.

Parameter	Mode	Nb laboratories
Method	AFNOR BKR 23/02-11/02 (Compass L. mono)	63
	ISO/NF EN ISO 11290-1	57
	AFNOR AES 10/03-09/00 (ALOA one day)	57
	NM ISO 11290-1	29
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	24
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	8
	AFNOR BRD 07/16-01/09 (Agar Listeria)	7
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	7
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	5
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	5
	AFNOR BRD 07/10-04/05 (IQ Check Listeria)	5
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	4
	AFNOR UNI 03/04-04/05 (Listeria Precis)	4
	AFNOR UNI 03/08-11/13 (PCR)	2
	Internal method	1
	Other	3

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 BKR 23/02-11/02 **Compass L. mono**	Half-Fraser	30°C - 24±2h			Compass Listeria Agar 37°C – 24h
AFNOR AES 10/03-09/00 **ALOA one day**	Half-Fraser	30°C - 24±2h			ALOA One Day 37°C – 24/48h
AFNOR BRD 07/04-09/98 **Rapid' L. mono**	Half-Fraser	30°C - 24±2h			Rapid L'mono 37°C – 24h
AFNOR BRD 07/16-01/09 **Agar Listeria**	Half-Fraser	30°C - 24±2h			Agar Listeria 37°C – 24h
AFNOR BIO 12/11-03/04 **VIDAS LMO2 (37°C)**	Half-Fraser	30°C - 24/26h	Fraser	37°C - 24/26h	Chromogenic medium / Palcam / Oxford
AFNOR BIO 12/27-02/10 **VIDAS LMX**	LMX	37°C - 26/30h			ChromID 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 12/02-06/94 **VIDAS Listeria**	Half-Fraser	37°C - 26/30h	Fraser	30°C - 24/26h	Palcam et Oxford 37°C – 24h
AFNOR UNI 03/08-11/13 **PCR**	LEB	37°C - 24/28h			Lysis + PCR
AFNOR UNI 03/04-04/05 **Listeria Precis**	One Broth Listeria	30°C - 24±2h			Brilliance Listeria 37°C – 24h
AFNOR BIO 12/18-03/06 **VIDAS LDUO**	LX	30°C - 24±2h	LX	30°C - 24/26h	Chromogenic medium / Palcam / Oxford
AFNOR BRD 07/10-04/05 **IQ Check Listeria**	Half-Fraser / LSB	30°C – 23/25h			Lysis + PCR

The detail of the methodology followed by 86 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 methods, and the 4 laboratories using an internal or another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	57
	NM ISO 11290-1	29
	Internal method	1
	Other	3
Primary enrichment medium	None primary enrichment	1
	Half-Fraser	86
	One broth Listeria	1
	Other	2
Primary enrichment temperature	30°C	83
	37°C	6
	23°C	1
Primary enrichment duration	18-28 h	88
	48 h	2
Secondary enrichment medium	None secondary enrichment	6
	Fraser	83
	Other	1
Secondary enrichment temperature	37±1°C	81
	30°C	3
Secondary enrichment duration	22-24 h	71
	48 h	14
Isolation medium	Palcam	64
	Ottaviani et Agosti	48
	Compass Listeria	37
	Oxford	15
	Rapid L'mono	3
	Brilliance Listeria	1
Isolation temperature	37±1°C	88
	30°C	1
Isolation duration	48±1 h	62
	23-24 h	27
Confirmation test	None	3
	Biochemical	60
	Biochemical + CAMP	20
	MALDI-TOF mass spectrometry	5
Nb of colonies per plate	1	22
	2-4	10
	5	44

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 is used to assess the trueness, the reference standard deviation is used for the assessment of the precision ; those are consensual values obtained with the results of all the participants. These values are obtained by robust estimation methods 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 x 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 been 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, dilution) 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 NF 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 NF 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 NF 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.

The assigned value uncertainty is calculated with the following formula :

$$u(Xpt) = 1{,}25 \times \frac{\sigma pt}{\sqrt{p}}$$

with σ_{pt}, robust standard deviation (standard deviation for proficiency assessment) and p, number of laboratories.

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 NF ISO 13528 specifies that:
- |z| ≤ 2,0 is considered as satisfactory,
- 2,0 < |z| < 3,0 is considered as a warning signal,
- |z| ≥ 3,0 is considered as an action signal (or not acceptable).

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)	5.376
Assigned value uncertainty (log cfu/g)	0.0052
Standard deviation for proficiency assessment (log cfu/g)	0.0699
Standard deviation for precision (log cfu/g)	0.0496
Interlaboratory's standard deviation (log cfu/g)	0.0663
Reproducibility standard deviation (log cfu/g)	0.0828

3.1.2. ENTEROBACTERIACEAE

A significant "effect" of the culture medium, 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 two groups :

Enterobacteriaceae	Group 1	Group 2
Assigned value of the contamination (log cfu/g)	2.671	3.018
Assigned value uncertainty (log cfu/g)	0.0176	0.0262
Standard deviation for proficiency assessment (log cfu/g)	0.2060	0.1391
Standard deviation for precision (log cfu/g)	0.1019	
Interlaboratory's standard deviation (log cfu/g)	0.1975	0.1260
Reproducibility standard deviation (log cfu/g)	0.2222	0.1620

Comment : Due to the homogeneity's issue between contaminated units, units 1 and 3 were not retained in the statistical analysis.

Unit1 : Assigned value of the contamination (log cfu/g) = 2.808
Standard deviation for proficiency assessment (log cfu/g) = 0.2358

Unité 3 : Assigned value of the contamination (log cfu/g) = 2.801
Standard deviation for proficiency assessment (log cfu/g) = 0.2291

3.1.3. TOTAL COLIFORMS

None significant effect of the analysis technique has been highlighted.

Total coliforms	
Assigned value of the contamination (log cfu/g)	2.622
Assigned value uncertainty (log cfu/g)	0.0175
Standard deviation for proficiency assessment (log cfu/g)	0.1973
Standard deviation for precision (log cfu/g)	0.1033
Interlaboratory's standard deviation (log cfu/g)	0.1919
Reproducibility standard deviation (log cfu/g)	0.2179

3.1.4. THERMOTOLERANT COLIFORMS

None significant effect of the analysis technique has been highlighted.

Thermotolerant coliforms	
Assigned value of the contamination (log cfu/g)	2.584
Assigned value uncertainty (log cfu/g)	0.0173
Standard deviation for proficiency assessment (log cfu/g)	0.1841
Standard deviation for precision (log cfu/g)	0.1054
Interlaboratory's standard deviation (log cfu/g)	0.1738
Reproducibility standard deviation (log cfu/g)	0.2014

Comment : Due to the homogeneity's issue between contaminated units, units 1 and 3 were not retained in the statistical analysis.
Unit1 : Assigned value of the contamination (log cfu/g) = 2.670
Standard deviation for proficiency assessment (log cfu/g) = 0.1924
Unité 3 : Assigned value of the contamination (log cfu/g) = 2.651
Standard deviation for proficiency assessment (log cfu/g) = 0.1885

3.1.5. *ESCHERICHIA COLI*

A significant "effect" of the culture medium, manufacturer and the preparation mode of the culture medium 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)	2.523
Assigned value uncertainty (log cfu/g)	0.0130
Standard deviation for proficiency assessment (log cfu/g)	0.1699
Standard deviation for precision (log cfu/g)	0.1014
Interlaboratory's standard deviation (log cfu/g)	0.1638
Reproducibility standard deviation (log cfu/g)	0.1926

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°1, 3, 4 and 5 were artificially contaminated.

A significant "effect" of the size of the test sample, the preparation of the initial suspension and the retained dilution 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 :

Anaerobic sulfite-reducing bacteria	
Assigned value of the contamination (log cfu/g)	3.032
Assigned value uncertainty (log cfu/g)	0.0172
Standard deviation for proficiency assessment (log cfu/g)	0.2008
Standard deviation for precision (log cfu/g)	0.0856
Interlaboratory's standard deviation (log cfu/g)	0.1961
Reproducibility standard deviation (log cfu/g)	0.2140

Comment :
- 11 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 270 to 25000 cfu/g.

3.1.7. *CLOSTRIDIUM PERFRINGENS*

Only units n°1; 3, 4 and 5 were artificially contaminated.

A significant "effect" of the retained dilution 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 :

Clostridium perfringens	
Assigned value of the contamination (log cfu/g)	3.011
Assigned value uncertainty (log cfu/g)	0.0186
Standard deviation for proficiency assessment (log cfu/g)	0.1980
Standard deviation for precision (log cfu/g)	0.0899
Interlaboratory's standard deviation (log cfu/g)	0.1928
Reproducibility standard deviation (log cfu/g)	0.2128

Comment :
- 5 laboratories detected *C. perfringens* in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 54 to 2000 cfu/g.

3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

A significant "effect" of the size of the test sample 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)	3.811
Assigned value uncertainty (log cfu/g)	0.0097
Standard deviation for proficiency assessment (log cfu/g)	0.1271
Standard deviation for precision (log cfu/g)	0.0594
Interlaboratory's standard deviation (log cfu/g)	0.1243
Reproducibility standard deviation (log cfu/g)	0.1377

3.1.9. *LISTERIA MONOCYTOGENES*

Only units n°1 and 3 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

Listeria monocytogenes	
Assigned value of the contamination (log cfu/g)	3.771
Assigned value uncertainty (log cfu/g)	0.0081
Standard deviation for proficiency assessment (log cfu/g)	0.0976
Standard deviation for precision (log cfu/g)	0.0622
Interlaboratory's standard deviation (log cfu/g)	0.0872
Reproducibility standard deviation (log cfu/g)	0.1071

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 units n°1 and 3 were artificially contaminated.
296 laboratories obtained correct results.
8 laboratories obtained false positive results (respectively 1, 7 and 1 false-positive for units n° 2, 4 and 5).
3 laboratories obtained false negative results (respectively 1 and 3 false-negative for units n° 1 and 3).

3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

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

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 58th scheme.

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

- Just one overtaking of the action limit ($z \leq -3.0$ or $z \geq 3.0$),
- 2 consecutives z scores out of 3 overtaking of the warning limit ($2.0 < z$ or $z < -2.0$),
- 6 consecutives z scores either positive or negative.

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