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PROFICIENCY TEST « RAEMA »

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

POWDER SCHEME N° 80
(11th MARCH 2025)
GENERAL REPORT

« The general report is public, it is available on the Website of ASA, results and informations are anonymous, they do not contain any confidential information »

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1- GENERAL DATA

1.1. PARTICIPATING LABORATORIES

329 laboratories participated to the 80th scheme. The sending was made on Tuesday 11th March 2025. We received **325** answers (98.8%).

1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+15	J0+16
Nb of laboratories	10	197	34	43	2	1	10	10	7	4	2	4	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

The sample included :
- one strain of *Enterococcus sp*. at a concentration level of 1.10^5 cfu/g in 5 units ;
- one strain of *Citrobacter sp.* at a concentration level of 1.10^3 cfu/g in 5 units ;
- one strain of *Serratia liquefaciens* at a concentration level of 6.10^2 cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of 4.10^2 cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of 2.10^3 cfu/g in 3 units ;
- one strain of *Staphylococcus aureus* at a concentration level of 5.10^3 cfu/g in 5 units ;
- one strain of *Salmonella* Anatum at a concentration level of 50 cfu/g in 2 units ;
- one strain of *Listeria monocytogenes* at a concentration level of 1.10^3 cfu/g in 4 units.

Samples have been prepared between January and March 2025. The maintenance of bacterial strains and check of their contamination are entrusted to an external provider.

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 17, 24 and 31 March 2025. These checks were realized by an external provider 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*.

1.4. EXECUTION OF ANALYZES

1.4.1. DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

355 laboratories (100%) specified it.

Beginning of analysis	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+13	J0+14	J0+15	J0+16
Nb of laboratories	1	34	34	18	7	1	123	55	22	6	4	14	1	3	2

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

325 laboratories (100%) specified it. The average temperature is **4.1°C** with a standard deviation of 1.1°C. The given data 20, 20.3, 21, 22, 22.1 and 25°C given by 8 laboratories were not taken into account for this calculation.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF THE SAMPLES

323 laboratories specified it (99.4%).
The average size is **17.4 g** with a standard deviation of 7.9 g. The minimum size indicated is 1 g and the maximum one is 30 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

For **324** answers (99.7%) :
210 laboratories (64.6%) prepare the initial suspension with adding diluent to powder.
113 laboratories (34.8%) prepare the initial suspension with adding powder to diluent.
1 laboratory (0.3%) used another technique to prepare the initial suspension.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

For **320** answers (98.5%) :
280 laboratories (86.1%) use Buffered Peptone Water (or equivalent) for the initial suspension.
32 laboratories (9.9%) use Peptone salt for the initial suspension.
8 laboratories (2.5%) used another diluent for the initial suspension.

2.4. HOMOGENEIZATION TECHNIQUE

For **323** answers (99.4%) :
291 laboratories (89.5%) homogeneize their sampling with a StomacherND.
21 laboratories (6.5%) used a manual homogenization.
9 laboratories (2.8%) used a Vortex mixer.
2 laboratories (0.6%) used another technique.

2.5. RESUSCITATION'S CONDITIONS

2.5.1. DURATION

312 laboratories (96.0%) specified it.
The average duration is **26.1 min** with a standard deviation of 15.8 min. The data 180 min given by two laboratories was not taken into account for this calculation.

2.5.2. TEMPERATURE

311 laboratories (95.7%) specified it.
The average temperature is **21.4°C** with a standard deviation of 3.6°C.

2.6. MICROORGANISM AT 30°C

313 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 4833-1 (+A1)	197
	AFNOR 3M-01/1-09/89	40
	NM ISO 4833-1	30
	ISO/NF EN ISO 4833-2 (+A1)	15
	Internal method	9
	AFNOR BIO-12/35-05/13	8
	XP V08-034	6
	Other	8
	+ Spiral metho	20
Culture medium	Plate Count Agar	236
	Neogen® Petrifilms®	41
	Plate Count Agar + Milk	24
	Tempo AC	8
	Other	4
Preparation	Home made	108
	Ready to use not pre-poured	137
	Ready to use, plate, film, card	66
Plating method	Surface	60
	Pour	238
	Transfer Tempo filler®	8
1st dilution retained	- 1	12
	- 2	14
	- 3	267
	- 4	11
	- 5	2
	1/400	5
Incubation temperature	30°C	306
	32-33°C	3
	37°C	4
Incubation duration	69-73 h	269
	40-48 h	41
	24-26 h	2
	120 h	1

2.7. ENTEROBACTERIACEAE

284 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-054	102
	→ *NM 08.0.109*(1)	15
	ISO/NF EN ISO 21528-2	84
	AFNOR 3M-01/6-09/97	41
	NM ISO 21528-2	21
	AFNOR BRD-07/24-11/13	8
	AFNOR AES-10/07-01/08	5
	AFNOR BIO-12/21-12/06	4
	Internal method	4
	Other	0
Culture medium	VRBG	218
	Neogen® Petrifilms®	44
	Rapid'Enterobacteriaceae	10
	Rebecca	7
	Tempo EB	4
	Other	1
Preparation	Home made	89
	Ready to use not pre-poured	146
	Ready to use, plate, film, card	48
1st dilution retained	- 1	211
	- 2	69
	- 3	1
	1/400	1
Incubation temperature	37-37.5°C	194
	30-32°C	86
	35°C	4
Incubation duration	21-25 h	281
	48 h	3
Confirmatory test	Yes	87
	No	188

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

2.8. TOTAL COLIFORMS

217 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-050	101
	→ *NM 08.0.142*(2)	8
	ISO/NF ISO 4832	59
	NM ISO 4832	26
	AFNOR 3M	12
	Internal method	4
	AFNOR BRD-07/08-12/04	3
	AFNOR BIO-12/17-12/05	2
	Other	2
Culture medium	VRBL	195
	Neogen® Petrifilms®	13
	Rapid Ecoli 2	6
	Tempo TC	2
	Other	1
Preparation	Home made	89
	Ready to use not pre-poured	112
	Ready to use, plate, film, card	15
1st dilution retained	-1	201
	-2	14
Incubation temperature	30-32°C	203
	37°C	13
	44°C	1
Incubation duration	21-24 h	213
	48 h	4

AFNOR 3M method including :
1 laboratory specified utilization of AFNOR 3M-01/02-09/89 A method.
1 laboratory specified utilization of AFNOR 3M-CC 30°C method.

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

2.9. THERMOTOLERANT COLIFORMS

193 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-060	130
	→ *NM 08.0.124*(3)	33
	AFNOR 3M	16
	ISO/NF ISO 4832	10
	Internal method	2
	Other	2
Culture medium	VRBL	173
	Neogen® Petrifilms®	16
	Other	4
Preparation	Home made	85
	Ready to use not pre-poured	92
	Ready to use, plate, film, card	16
1st dilution retained	-1	177
	-2	15
Incubation temperature	42-44.5°C	190
	37°C	2
	30°C	1
Incubation duration	22-24 h	191
	48 h	2

AFNOR 3M method including :

2 laboratories 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-high sensitivity method.

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

2.10. ESCHERICHIA COLI

289 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 16649-2	173
	AFNOR 3M	39
	NM ISO 16649-2	30
	AFNOR BRD-07/01-07/93	16
	AFNOR BIO-12/13-02/05	8
	NM 08.0.108	5
	Internal method	4
	AFNOR AES-10/06-01/08	3
	AFNOR BIO-12/05-01/99	3
	AFNOR BRD-07/07-12/04	3
	ISO/NF EN ISO 16649-3	1
	Other	3
Culture medium	TBX	207
	Neogen® Petrifilms®	40
	Rapid E. coli 2	21
	Tempo EC	8
	Rebecca	6
	Coli ID	5
	Other	1
Preparation	Home made	90
	Ready to use not pre-poured	149
	Ready to use, plate, film, card	48
Plating method	Surface	42
	Pour	235
	Transfer Tempo filler®	8
1st dilution retained	-1	273
	-2	9
	-3	1
	1/400	4
Incubation temperature	40-48°C	265
	37°C	22
	30°C	1
Incubation duration	18-25 h	282
	48 h	4
	72 h	1
	Other	1

2.11. ANAEROBIC SULFITE-REDUCING BACTERIA

230 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-061	141
	→ *NM 08.0.125*[4]	19
	ISO/NF ISO 15213-1	44
	NM ISO 15213-1	13
	Internal method	7
	Other	5
Culture medium	TSC	196
	Iron Sulfite agar	27
	TSN	6
Preparation	Home made	86
	Ready to use not pre-poured	123
	Ready to use, plate, film, card	21
Seeding way	Plates	163
	Tubes	67
1st dilution retained	-1	65
	-2	148
	-3	15
Incubation temperature	44-49°C	167
	37°C	63
Incubation duration	16-24 h	185
	46-48 h	41
	72 h	4

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

2.12. CLOSTRIDIUM PERFRINGENS

198 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 15213-2	94
	ISO/NF EN ISO 7937	62
	NM ISO 15213-2	24
	NM ISO 7937	9
	Internal method	1
	Other	8
Culture medium	TSC	195
	Other	3
Preparation	Home made	68
	Ready to use not pre-poured	123
	Ready to use, plate, film, card	6
1st dilution retained	-1	61
	-2	137
Incubation temperature	36-37.5°C	191
	44-46°C	7
Incubation duration	17-24 h	190
	48 h	8
Confirmation test	None	29
	SIM agar	83
	Lactose-sulfite	59
	Acid phosphatase	8
	MALDI-TOF mass spectrometry	4
	Strip	2
	Other	3

2.13. COAGULASE POSITIVE STAPHYLOCOCCI

288 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 6888-2 (+A1)	127
	ISO/NF EN ISO 6888-1 (+A1)	73
	AFNOR BKR-23/10-12/15	27
	NM ISO 6888-1	25
	AFNOR 3M-01/09-04/03	10
	Internal method	6
	AFNOR BIO-12/28-04/10	5
	NM ISO 6888-2	4
	NM 08.0.112	3
	ISO/NF EN ISO 6888-3	3
	NordVal No :049	2
	Other	3
Culture medium	RPF	122
	BP+egg yolk tellurite	98
	Easy Staph	31
	BP+egg yolk tellurite+ sulfamethazine	12
	Neogen® Petrifilm®	11
	Tempo STA	5
	Rapid Staph	5
	Other	4
Preparation	Home made	81
	Ready to use not pre-poured	120
	Ready to use, plate, film, cards	87
Plating method	Surface	154
	Pour	123
	Transfer Tempo filler®	5
1st dilution retained	-1	121
	-2	161
	-3	3
	1/400	1
Incubation temperature	35-37°C	287
	30°C	1
Incubation duration	42-48.5 h	204
	20-25 h	83
	32 h	1
Confirmation test	None	172
	Staphylo-coagulase	87
	Clumping factor	15
	DNase	6
	MALDI-TOF mass spectrometry	4
	Other	3

2.14. LISTERIA MONOCYTOGENES – ENUMERATION

235 laboratories performed the enumeration.

RESUSCITATION

68 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	ISO/NF EN ISO 11290-2	70
	AFNOR BKR-23/05-12/07	54
	AFNOR AES-10/05-09/06	52
	NM ISO 11290-2	27
	AFNOR BRD-07/05-09/01	21
	AFNOR BRD-07/17-01/09	8
	Internal method	1
	Other	2
Resuscitation medium	Buffered Peptone Water or equivalent	200
	Half-fraser	30
	Fraser base	2
	Other	3
Enumeration medium	ALOA Count	106
	Compass Listeria	82
	Rapid Lmono	22
	AL Agar	15
	Palcam	4
	OCLA	2
	Other	3
Preparation	Home made	50
	Ready to use not pre-poured	43
	Ready to use, plate, film, card	141
Plating method	Surface	195
	Pour	39

Parameters	Mode	Nb laboratories
1st dilution retained	-1	199
	-2	32
Incubation temperature	37-37.5°C	231
	30°C	4
Incubation duration	44-48 h	199
	24 h	35
	34 h	1
Confirmation test	None	44
	Biochemical	139
	Biochemical + CAMP	31
	MALDI-TOF mass spectrometry	10
	Other	4
Nb of colonies tested per plate	1	52
	2-3	10
	5	115
	15	1
	150	1

2.15. SALMONELLA – DETECTION

300 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 (+A1)	86
	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	66
	NM ISO 6579-1	38
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	33
	AFNOR BIO 12/41-03/17 (SALMA One day)	18
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	18
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	10
	AFNOR BIO 12/01-04/94 (VIDAS SLM)	9
	AFNOR UNI 03/06-12/07 (Salmonella precis)	4
	AFNOR BIO 12/38-06/16 (GENE UP Salmonella)	4
	AFNOR BRD 07/06-07/04 (PCR)	4
	AFNOR UNI 03/07-11/13 (PCR)	3
	Internal method	2
	TRANSIA PLATE Salmonella GOLD	1
	Other	4

No detail of methodology was asked to laboratories using other methods than ISO/NF EN ISO 6579-1 (+A1) 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 supplement / 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
AFNOR BIO 12/32-10/11 **VIDAS SPT**		BPW + Salmonella supplement / 41,5°C - 18/24h	Chrom ID / 37°C - 24h
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 03/06-12/07 **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 UNI 03/07-11/13 **PCR**		BPW + supplement / 34-38°C – 20/24h	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 124 laboratories using ISO/NF EN ISO 6579-1 (+A1) and NM ISO 6579-1 methods, and the 6 laboratories using an internal or another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 6579-1 (+A1)	86
	NM ISO 6579-1	38
	Internal method	2
	Other	4
Pre-enrichment medium	None pre-enrichment	2
	Buffered Peptone Water	123
	Other	4
Pre-enrichment temperature	35-37°C	122
	41-42.5°C	4
	22°C	2
Pre-enrichment duration	16-20 h	90
	22-25 h	38
Enrichment medium	None enrichment	3
	RVS	121
	MKTTn	115
	Selenite-cystine broth	28
	Other	4
Isolation medium	XLD	117
	Hektoen	35
	Bismuth Sulfate	30
	GVB	19
	IRIS Salmonella agar	15
	ASAP	13
	SS	10
	Rapid Salmonella	9
	Compass Salmonella	4
	Rambach	3
	Brilliance Salmonella	2
	Other	10
Confirmation test	Biochemical	38
	Biochemical + serological agglutination	78
	MALDI-TOF mass spectrometry	7
	Other	2

2.16. LISTERIA MONOCYTOGENES – DETECTION

271 laboratories performed the detection.

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	66
	AFNOR BKR 23/02-11/02 (Compass L. mono)	65
	AFNOR AES 10/03-09/00 (ALOA one day)	43
	NM ISO 11290-1	28
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	19
	AFNOR BRD 07/16-01/09 (Agar Listeria)	10
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	7
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	5
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	5
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	5
	AFNOR BRD 07/10-04/05 (IQ Check Listeria)	3
	AFNOR UNI 03/04-04/05 (Listeria Precis)	3
	Internal method	3
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	3
	AFNOR UNI 03/08-11/13 (PCR)	2
	Other	4

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 12/27-02/10 **VIDAS LMX**	LMX	37°C - 26/30h			ChromID 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 12/02-06/94 **VIDAS Listeria**	Half-Fraser	37°C - 26/30h	Fraser	30°C - 24/26h	Palcam et Oxford 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 BRD 07/10-04/05 **IQ Check Listeria**	Half-Fraser / LSB	30°C – 23/25h			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 UNI 03/08-11/13 **PCR**	LEB	37°C - 24/28h			Lysis + PCR

The detail of the methodology followed by 94 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 methods, and the 7 laboratories using an internal or another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	66
	NM ISO 11290-1	28
	Internal method	3
	Other	4
Primary enrichment medium	None primary enrichment	1
	Half-Fraser	92
	One Broth Listeria	1
	Other	6
Primary enrichment temperature	30-32°C	91
	35-37°C	9
Primary enrichment duration	18-26 h	97
	28-30 h	2
	35 h	1
Secondary enrichment medium	None secondary enrichment	8
	Fraser	92
Secondary enrichment temperature	37±1°C	89
	30°C	3
Secondary enrichment duration	20-27 h	81
	48 h	11
Isolation medium	Palcam	68
	Ottaviani et Agosti	51
	Compass Listeria	38
	Oxford	15
	Rapid L'mono	7
	Brilliance Listeria	2
	Other	2
Isolation temperature	37±1°C	98
	30°C	1
Isolation duration	44-48 h	68
	22-24 h	31
Confirmation test	None	5
	Biochemical	59
	Biochemical + CAMP	29
	MALDI-TOF mass spectrometry	5
Nb of colonies per plate	1	25
	2-3	9
	5	50

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:
- $|z| \leq 2{,}0$ is considered as satisfactory (acceptable),
- $2{,}0 < |z| < 3{,}0$ is considered as a warning signal,
- $|z| \geq 3{,}0$ 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)	5.010
Assigned value uncertainty (log cfu/g)	0.0052
Standard deviation for proficiency assessment (log cfu/g)	0.0685
Standard deviation for precision (log cfu/g)	0.0537
Interlaboratory's standard deviation (log cfu/g)	0.0641
Reproducibility standard deviation (log cfu/g)	0.0836

3.1.2. ENTEROBACTERIACEAE

A significant "effect" of the culture medium, the 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 (221 laboratories)	Group 2 (20 laboratories)	Group 3 (43 laboratories)
Assigned value of the contamination (log cfu/g)	2.812	3.037	3.253
Assigned value uncertainty (log cfu/g)	0.0169	0.0432	0.0236
Standard deviation for proficiency assessment (log cfu/g)	0.1858	0.1505	0.1223
Standard deviation for precision (log cfu/g)	0.0808		
Interlaboratory's standard deviation (log cfu/g)	0.1823	0.1461	0.1168
Reproducibility standard deviation (log cfu/g)	0.1994	0.1670	0.1421

3.1.3. TOTAL COLIFORMS

A significant "effect" of the method, the culture medium, the manufacturer 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 :

Total coliforms	
Assigned value of the contamination (log cfu/g)	2.774
Assigned value uncertainty (log cfu/g)	0.0190
Standard deviation for proficiency assessment (log cfu/g)	0.2073
Standard deviation for precision (log cfu/g)	0.0807
Interlaboratory's standard deviation (log cfu/g)	0.2041
Reproducibility standard deviation (log cfu/g)	0.2195

3.1.4. THERMOTOLERANT COLIFORMS

A significant “effect” of 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 :

Thermotolerant coliforms	Group 1 (178 laboratories)	Group 2 (15 laboratories)
Assigned value of the contamination (log cfu/g)	2.739	3.132
Assigned value uncertainty (log cfu/g)	0.0169	0.0661
Standard deviation for proficiency assessment (log cfu/g)	0.1653	0.1977
Standard deviation for precision (log cfu/g)	0.0830	
Interlaboratory’s standard deviation (log cfu/g)	0.1611	0.1942
Reproducibility standard deviation (log cfu/g)	0.1802	0.2104

Comment : Due to the low number of laboratories included in group 2, the assigned value uncertainty is not insignificant (cf NF ISO 13528 §9.2.1). Laboratories included in the group 2 obtain a satisfactory z-score (without impact), except one laboratory with an advertisement signal. This laboratory has been warned.

3.1.5. *ESCHERICHIA COLI*

None significant effect of the analysis technique has been highlighted.

Escherichia coli	
Assigned value of the contamination (log cfu/g)	2.638
Assigned value uncertainty (log cfu/g)	0.0118
Standard deviation for proficiency assessment (log cfu/g)	0.1512
Standard deviation for precision (log cfu/g)	0.0811
Interlaboratory’s standard deviation (log cfu/g)	0.1468
Reproducibility standard deviation (log cfu/g)	0.1677

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°2, 3 and 5 were artificially contaminated.

A significant “effect” of the manufacturer of the culture medium, the seeding way 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)	3.316
Assigned value uncertainty (log cfu/g)	0.0188
Standard deviation for proficiency assessment (log cfu/g)	0.2156
Standard deviation for precision (log cfu/g)	0.0836
Interlaboratory’s standard deviation (log cfu/g)	0.2102
Reproducibility standard deviation (log cfu/g)	0.2262

Comment :
- 3 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 580 to 1200 cfu/g.
- 3 laboratories detected ASR in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 10 to 1500 cfu/g.

3.1.7. *CLOSTRIDIUM PERFRINGENS*

Only units n°2, 3 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)	3.336
Assigned value uncertainty (log cfu/g)	0.0187
Standard deviation for proficiency assessment (log cfu/g)	0.1982
Standard deviation for precision (log cfu/g)	0.0808
Interlaboratory’s standard deviation (log cfu/g)	0.1926
Reproducibility standard deviation (log cfu/g)	0.2089

Comment :
- 2 laboratories detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 190 to 3900 cfu/g.
- None laboratory detected *C. perfringens* in unit n°4 non-artificially contaminated by *C. perfringens*.

3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

A significant “effect” of the preparation 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 :

Coagulase positive Staphylococci	
Assigned value of the contamination (log cfu/g)	3.782
Assigned value uncertainty (log cfu/g)	0.0101
Standard deviation for proficiency assessment (log cfu/g)	0.1288
Standard deviation for precision (log cfu/g)	0.0596
Interlaboratory’s standard deviation (log cfu/g)	0.1260
Reproducibility standard deviation (log cfu/g)	0.1394

3.1.9. *LISTERIA MONOCYTOGENES*

Only units n°1, 2, 3 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 :

Listeria monocytogenes	
Assigned value of the contamination (log cfu/g)	3.135
Assigned value uncertainty (log cfu/g)	0.0073
Standard deviation for proficiency assessment (log cfu/g)	0.0856
Standard deviation for precision (log cfu/g)	0.0702
Interlaboratory’s standard deviation (log cfu/g)	0.0780
Reproducibility standard deviation (log cfu/g)	0.1050

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°2 and 5 were artificially contaminated.
289 laboratories obtained correct results.
10 laboratories obtained false positive results (respectively 6, 7 and 3 false-positive for units n° 1, 3 and 4).
7 laboratories obtained false negative results (respectively 5 and 5 false-negative for units n° 2 and 5).

3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

Only units n°1, 2, 3 and 5 were artificially contaminated.
265 laboratories obtained correct results.
4 laboratories obtained false positive results (respectively 4 for unit n° 4).
5 laboratories obtained false negative results (respectively 1, 3, 3 and 2 false-negative for units n°1, 2, 3, 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 60th 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.