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PROFICIENCY TEST « RAEMA »

POWDER SCHEME N° 79
(1st OCTOBER 2024)
GENERAL REPORT

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Table of contents

1- GENERAL DATA

1.1. PARTICIPATING LABORATORIES

338 laboratories participated to the 79th scheme. The sending was made on Tuesday 1st October 2024. We received **335** answers (99.1%).

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+13	J0+14	J0+20
Nb of laboratories	12	211	46	26	1	1	10	8	7	1	5	1	3	3

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 2.10^3 cfu/g in 5 units ;
- one strain of *Serratia liquefaciens* at a concentration level of 2.10^3 cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of 2.10^3 cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of 1.10^3 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 50 cfu/g in 3 units ;
- one strain of *Listeria monocytogenes* at a concentration level of $1,5.10^3$ cfu/g in 4 units.

Samples have been prepared between August and October 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 7, 14 and 21 October 2024. 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*.

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+6	J0+7	J0+8	J0+9	J0+10	J0+13	J0+14	J0+15	J0+20	J0+21
Nb of laboratories	1	39	36	14	1	141	57	16	6	2	16	1	2	2	1

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

334 laboratories (99.7%) specified it. The average temperature is **4.0°C** with a standard deviation of 1.4°C. The given data 20, 21, 22, 25, 29 and 30°C given by 11 laboratories were not taken into account for this calculation.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF THE SAMPLES

333 laboratories specified it (99.4%).
The average size is **17.7 g** with a standard deviation of 8.1 g. The minimum size indicated is 1 g and the maximum one is 100 g (data not taken into account for this calculation).

2.2. PREPARATION OF THE INITIAL SUSPENSION

For **334** answers (99.7%) :
208 laboratories (62.1%) prepare the initial suspension with adding diluent to powder.
125 laboratories (37.3%) 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 **330** answers (98.5%) :
290 laboratories (86.6%) use Buffered Peptone Water (or equivalent) for the initial suspension.
35 laboratories (10.4%) use Peptone salt for the initial suspension.
5 laboratories (1.5%) used another diluent for the initial suspension.

2.4. HOMOGENEIZATION TECHNIQUE

For **333** answers (99.4%) :
296 laboratories (88.3%) homogeneize their sampling with a StomacherND.
27 laboratories (8.1%) used a manual homogenization.
7 laboratories (2.1%) used a Vortex mixer.
3 laboratories (0.9%) used another technique.

2.5. RESUSCITATION'S CONDITIONS

2.5.1. DURATION

317 laboratories (94.6%) specified it.
The average duration is **26.6 min** with a standard deviation of 15.1 min. The data 120 min given by one laboratory was not taken into account for this calculation.

2.5.2. TEMPERATURE

317 laboratories (94.6%) specified it.
The average temperature is **21.6°C** with a standard deviation of 3.3°C.

2.6. MICROORGANISM AT 30°C

316 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 4833-1 (+A1)	189
	AFNOR 3M-01/1-09/89	42
	NM ISO 4833-1	28
	ISO/NF EN ISO 4833-2 (+A1)	16
	AFNOR BIO-12/35-05/13	14
	Internal method	9
	XP V08-034	9
	Other	9
	+ Spiral metho	23
Culture medium	Plate Count Agar	226
	Neogen® Petrifilms®	46
	Plate Count Agar + Milk	26
	Tempo AC	14
	Other	3
Preparation	Home made	110
	Ready to use not pre-poured	128
	Ready to use, plate, film, card	76
Plating method	Surface	66
	Pour	230
	Transfer Tempo filler®	14
1st dilution retained	- 1	10
	- 2	15
	- 3	254
	- 4	18
	- 5	2
	- 6	2
	1/400	6
	1/4000	6
Incubation temperature	30°C	311
	33-35°C	2
	37°C	2
	25°C	1
Incubation duration	69-72 h	262
	40-48 h	50
	24-26 h	2
	120 h	2

2.7. ENTEROBACTERIACEAE

280 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-054	107
	→ *NM 08.0.109*(1)	18
	ISO/NF EN ISO 21528-2	67
	AFNOR 3M-01/6-09/97	42
	NM ISO 21528-2	12
	AFNOR BIO-12/21-12/06	12
	AFNOR AES-10/07-01/08	7
	AFNOR BRD-07/24-11/13	7
	Internal method	5
	Other	2
Culture medium	VRBG	204
	Neogen® Petrifilms®	45
	Tempo EB	12
	Rebecca	8
	Rapid'Enterobacteriaceae	8
	Other	1
Preparation	Home made	90
	Ready to use not pre-poured	129
	Ready to use, plate, film, card	60
1st dilution retained	- 1	87
	- 2	177
	- 3	4
	1/40	1
	1/400	9
Incubation temperature	37°C	181
	30-32°C	85
	35°C	14
Incubation duration	22-24 h	274
	48 h	6
Confirmatory test	Yes	69
	No	207

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

2.8. TOTAL COLIFORMS

218 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-050	104
	→ *NM 08.0.142*(2)	9
	ISO/NF ISO 4832	51
	NM ISO 4832	22
	AFNOR 3M	16
	AFNOR BIO-12/17-12/05	5
	AFNOR BRD-07/08-12/04	3
	Internal method	3
	Other	3
Culture medium	VRBL	187
	Neogen® Petrifilms®	17
	Rapid Ecoli 2	5
	Tempo TC	5
	Other	3
Preparation	Home made	89
	Ready to use not pre-poured	106
	Ready to use, plate, film, card	22
1st dilution retained	-1	81
	-2	128
	-3	1
	1/40	3
	1/400	2
Incubation temperature	30°C	203
	35-37°C	14
Incubation duration	21-25 h	213
	48 h	4

AFNOR 3M method including :
1 laboratory specified utilization of AFNOR 3M-01/06-09/97 method.
2 laboratories specified utilization of AFNOR 3M-01/02-09/89 A method.

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

2.9. THERMOTOLERANT COLIFORMS

199 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-060	133
	→ *NM 08.0.124*(3)	30
	AFNOR 3M	19
	ISO/NF ISO 4832	12
	Other	3
Culture medium	VRBL	174
	Neogen® Petrifilms®	19
	Other	5
Preparation	Home made	83
	Ready to use not pre-poured	95
	Ready to use, plate, film, card	18
1st dilution retained	-1	74
	-2	122
	-3	1
Incubation temperature	42-44.5°C	195
	37°C	4
Incubation duration	22-24 h	196
	48 h	3

AFNOR 3M method including :

4 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-Petrifilm coliforms method.

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

2.10. ESCHERICHIA COLI

292 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 16649-2	170
	AFNOR 3M	40
	NM ISO 16649-2	27
	AFNOR BRD-07/01-07/93	14
	AFNOR BIO-12/13-02/05	14
	AFNOR AES-10/06-01/08	6
	Internal method	6
	NM 08.0.108	4
	AFNOR BIO-12/05-01/99	3
	AFNOR BRD-07/07-12/04	3
	ISO/NF EN ISO 16649-3	2
	Other	2
Culture medium	TBX	203
	Neogen® Petrifilms®	41
	Rapid E. coli 2	18
	Tempo EC	14
	Rebecca	7
	Coli ID	5
	Glutamate + TBX	1
	Other	2
Preparation	Home made	89
	Ready to use not pre-poured	145
	Ready to use, plate, film, card	57
Plating method	Surface	42
	Pour	233
	Transfer Tempo filler®	14
1st dilution retained	-1	98
	-2	177
	-3	3
	1/40	5
	1/400	6
Incubation temperature	40-46°C	263
	37°C	27
	30°C	1
Incubation duration	18-25 h	288
	48 h	3

2.11. ANAEROBIC SULFITE-REDUCING BACTERIA

221 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-061	142
	→ *NM 08.0.125*(4)	17
	ISO/NF ISO 15213-1	37
	NM ISO 15213	7
	Internal method	6
	Other	9
Culture medium	TSC	194
	Iron Sulfite agar	17
	TSN	4
	Other	5
Preparation	Home made	78
	Ready to use not pre-poured	121
	Ready to use, plate, film, card	21
Seeding way	Plates	150
	Tubes	68
1st dilution retained	-1	107
	-2	106
	-3	4
	-4	1
Incubation temperature	44-46°C	160
	37°C	60
Incubation duration	16-24 h	178
	46-48 h	37
	72 h	5

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

2.12. CLOSTRIDIUM PERFRINGENS

190 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 15213-2	81
	ISO/NF EN ISO 7937	76
	NM ISO 7937	14
	NM 08.0.111	3
	Internal method	2
	Other	12
Culture medium	TSC	189
Preparation	Home made	60
	Ready to use not pre-poured	127
	Ready to use, plate, film, card	2
1st dilution retained	-1	95
	-2	92
	-3	1
Incubation temperature	37-37.5°C	185
	44-46°C	5
Incubation duration	18-24 h	186
	48 h	4
Confirmation test	None	31
	Lactose-sulfite	70
	SIM agar	63
	Acid phosphatase	10
	MALDI-TOF mass spectrometry	4
	Strip	2
	Other	1

2.13. COAGULASE POSITIVE STAPHYLOCOCCI

290 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 6888-2 (+A1)	137
	ISO/NF EN ISO 6888-1 (+A1)	62
	NM ISO 6888-1	22
	AFNOR BKR-23/10-12/15	21
	AFNOR BIO-12/28-04/10	15
	AFNOR 3M-01/9-04/03	11
	Internal method	6
	NM ISO 6888-2	4
	NM 08.0.112	3
	ISO/NF EN ISO 6888-3	2
	NordVal No :049	1
	Other	3
Culture medium	RPF	127
	BP+egg yolk tellurite	87
	Easy Staph	27
	Tempo STA	15
	Neogen® Petrifilm®	12
	BP+egg yolk tellurite+ sulfamethazine	12
	Rapid Staph	4
	Other	4
Preparation	Home made	77
	Ready to use not pre-poured	120
	Ready to use, plate, film, cards	91
Plating method	Surface	140
	Pour	131
	Transfer Tempo filler®	14
1st dilution retained	-1	120
	-2	151
	-3	4
	1/40	7
	1/400	5
Incubation temperature	35-37°C	285
	30-32.5°C	3
Incubation duration	42-48 h	201
	18-25 h	85
	32-34 h	2
Confirmation test	None	182
	Staphylo-coagulase	68
	Clumping factor	18
	DNase	8
	MALDI-TOF mass spectrometry	3
	Other	2

2.14. LISTERIA MONOCYTOGENES – ENUMERATION

238 laboratories performed the enumeration.

RESUSCITATION

74 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
	AFNOR BKR-23/05-12/07	63
	ISO/NF EN ISO 11290-2	54
	NM ISO 11290-2	23
	AFNOR BRD-07/05-09/01	23
	AFNOR BRD-07/17-01/09	9
	Other	2
Resuscitation medium	Buffered Peptone Water or equivalent	194
	Half-fraser	40
	Fraser base	2
	Other	1
Enumeration medium	ALOA Count	105
	Compass Listeria	89
	Rapid Lmono	23
	AL Agar	14
	Palcam	3
	OCLA	2
	Other	2
Preparation	Home made	39
	Ready to use not pre-poured	49
	Ready to use, plate, film, card	149
Plating method	Surface	189
	Pour	44

Parameters	Mode	Nb laboratories
1st dilution retained	-1	173
	-2	61
	-4	1
Incubation temperature	36-37.2°C	236
	30°C	2
Incubation duration	42-48 h	201
	22-24 h	37
Confirmation test	None	50
	Biochemical	139
	Biochemical + CAMP	32
	MALDI-TOF mass spectrometry	7
	Other	4
Nb of colonies tested per plate	1	58
	2-3	11
	5	108
	10	1
	300	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	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	74
	ISO/NF EN ISO 6579-1 (+A1)	72
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	35
	NM ISO 6579-1	32
	AFNOR BIO 12/41-03/17 (SALMA One day)	23
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	22
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	13
	AFNOR BIO 12/01-04/94 (VIDAS SLM)	7
	AFNOR UNI 03/06-12/07 (Salmonella precis)	3
	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)	3
	Internal method	2
	Other	6

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 UNI 03/07-11/13 **PCR**		BPW + supplement / 34-38°C – 20/24h	Lysis + PCR
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 104 laboratories using ISO/NF EN ISO 6579-1 (+A1) and NM ISO 6579-1 methods, and the 8 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)	72
	NM ISO 6579-1	32
	Internal method	2
	Other	6
Pre-enrichment medium	None pre-enrichment	1
	Buffered Peptone Water	109
	Other	2
Pre-enrichment temperature	35-37°C	109
	41-42.5°C	2
	22°C	1
Pre-enrichment duration	16-20 h	82
	22-24 h	29
Enrichment medium	None enrichment	3
	RVS	105
	MKTTn	98
	Selenite-cystine broth	25
	Other	4
Isolation medium	XLD	100
	Hektoen	31
	Bismuth Sulfate	26
	GVB	13
	IRIS Salmonella agar	12
	ASAP	10
	SS	10
	Rapid Salmonella	7
	Compass Salmonella	3
	Brilliance Salmonella	3
	Rambach	2
	Other	11
Confirmation test	Biochemical	37
	Biochemical + serological agglutination	64
	MALDI-TOF mass spectrometry	6
	Other	4

2.16. LISTERIA MONOCYTOGENES – DETECTION

274 laboratories performed the detection.

Parameter	Mode	Nb laboratories
Method	AFNOR BKR 23/02-11/02 (Compass L. mono)	66
	AFNOR AES 10/03-09/00 (ALOA one day)	57
	ISO/NF EN ISO 11290-1	45
	NM ISO 11290-1	28
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	23
	AFNOR BRD 07/16-01/09 (Agar Listeria)	10
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	8
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	7
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	6
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	6
	AFNOR BRD 07/10-04/05 (IQ Check Listeria)	5
	AFNOR UNI 03/04-04/05 (Listeria Precis)	4
	AFNOR UNI 03/08-11/13 (PCR)	3
	Internal method	3
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	1
	Other	2

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 12/02-06/94 **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 UNI 03/08-11/13 **PCR**	LEB	37°C - 24/28h			Lysis + PCR
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 72 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 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 11290-1	44
	NM ISO 11290-1	28
	Internal method	3
	Other	2
Primary enrichment medium	None primary enrichment	1
	Half-Fraser	72
	Other	4
Primary enrichment temperature	30-32°C	74
	35-37°C	3
Primary enrichment duration	21-26 h	75
	48 h	1
	35 h	1
Secondary enrichment medium	None secondary enrichment	5
	Fraser	71
Secondary enrichment temperature	37±1°C	68
	30°C	5
Secondary enrichment duration	20-25 h	62
	48 h	11
Isolation medium	Palcam	51
	Ottaviani et Agosti	33
	Compass Listeria	32
	Oxford	15
	Rapid L'mono	3
	Brilliance Listeria	2
	Other	4
Isolation temperature	35-37°C	73
	30°C	1
Isolation duration	46-48 h	47
	22-24 h	27
Confirmation test	None	3
	Biochemical	48
	Biochemical + CAMP	22
	MALDI-TOF mass spectrometry	1
	Other	1
Nb of colonies per plate	1	15
	2-3	5
	5	42
	10	1

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 (acceptable),
- 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)	5.044
Assigned value uncertainty (log cfu/g)	0.0059
Standard deviation for proficiency assessment (log cfu/g)	0.0780
Standard deviation for precision (log cfu/g)	0.0545
Interlaboratory's standard deviation (log cfu/g)	0.0741
Reproducibility standard deviation (log cfu/g)	0.0920

3.1.2. ENTEROBACTERIACEAE

A significant "effect" of the preparation mode of the culture medium 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 (52 laboratories)	Group 2 (72 laboratories)	Group 3 (156 laboratories)
Assigned value of the contamination (log cfu/g)	3.083	3.416	3.542
Assigned value uncertainty (log cfu/g)	0.0574	0.0209	0.0141
Standard deviation for proficiency assessment (log cfu/g)	0.2557	0.1348	0.1375
Standard deviation for precision (log cfu/g)		0.0744	
Interlaboratory's standard deviation (log cfu/g)	0.2535	0.1306	0.1334
Reproducibility standard deviation (log cfu/g)	0.2642	0.1503	0.1527

3.1.3. TOTAL COLIFORMS

A significant "effect" of the culture medium, the manufacturer, the preparation mode of the culture medium 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 (31 laboratories)	Group 2 (143 laboratories)	Group 3 (44 laboratories)
Assigned value of the contamination (log cfu/g)	3.045	3.378	3.568
Assigned value uncertainty (log cfu/g)	0.0582	0.0198	0.0190
Standard deviation for proficiency assessment (log cfu/g)	0.2031	0.1775	0.0997
Standard deviation for precision (log cfu/g)		0.0727	
Interlaboratory's standard deviation (log cfu/g)	0.2005	0.1745	0.0943
Reproducibility standard deviation (log cfu/g)	0.2138	0.1897	0.1201

3.1.4. THERMOTOLERANT COLIFORMS

A significant "effect" of the preparation mode of the culture medium 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 :

Thermotolerant coliforms	Group 1 (49 laboratories)	Group 2 (150 laboratories)
Assigned value of the contamination (log cfu/g)	3.078	3.441
Assigned value uncertainty (log cfu/g)	0.0505	0.0165
Standard deviation for proficiency assessment (log cfu/g)	0.2101	0.1583
Standard deviation for precision (log cfu/g)	0.0728	
Interlaboratory's standard deviation (log cfu/g)	0.2075	0.1549
Reproducibility standard deviation (log cfu/g)	0.2205	0.1719

3.1.5. *ESCHERICHIA COLI*

A significant "effect" of the preparation mode of the culture medium 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 :

Escherichia coli	Group 1 (51 laboratories)	Group 2 (241 laboratories)
Assigned value of the contamination (log cfu/g)	3.008	3.464
Assigned value uncertainty (log cfu/g)	0.0596	0.0117
Standard deviation for proficiency assessment (log cfu/g)	0.2611	0.1408
Standard deviation for precision (log cfu/g)	0.0745	
Interlaboratory's standard deviation (log cfu/g)	0.2590	0.1368
Reproducibility standard deviation (log cfu/g)	0.2695	0.1557

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°3 and 4 were artificially contaminated.

A significant "effect" of the preparation mode of the culture medium 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)	3.079
Assigned value uncertainty (log cfu/g)	0.0171
Standard deviation for proficiency assessment (log cfu/g)	0.1882
Standard deviation for precision (log cfu/g)	0.0859
Interlaboratory's standard deviation (log cfu/g)	0.1782
Reproducibility standard deviation (log cfu/g)	0.1978

Comment :
- 4 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 8 to 1000 cfu/g.
- 7 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 14 to 1500 cfu/g.
- 9 laboratories detected ASR in unit n°5 non-artificially contaminated by *C. perfringens* with a contamination level from 11 to 3500 cfu/g.

3.1.7. *CLOSTRIDIUM PERFRINGENS*

Only units n°3 and 4 were artificially contaminated.

A significant "effect" of the preparation mode of the initial suspension, the preparation mode of the culture medium 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 :

Clostridium perfringens	
Assigned value of the contamination (log cfu/g)	3.098
Assigned value uncertainty (log cfu/g)	0.0152
Standard deviation for proficiency assessment (log cfu/g)	0.1558
Standard deviation for precision (log cfu/g)	0.0908
Interlaboratory's standard deviation (log cfu/g)	0.1420
Reproducibility standard deviation (log cfu/g)	0.1685

Comment :
- 1 laboratory detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level of 20 cfu/g.
- 3 laboratories detected *C. perfringens* in unit n°5 non-artificially contaminated by *C. perfringens* with a contamination level from 800 to 1800 cfu/g.

3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

None significant effect of the analysis technique has been highlighted.

Coagulase positive Staphylococci	
Assigned value of the contamination (log cfu/g)	3.513
Assigned value uncertainty (log cfu/g)	0.0116
Standard deviation for proficiency assessment (log cfu/g)	0.1475
Standard deviation for precision (log cfu/g)	0.0756
Interlaboratory's standard deviation (log cfu/g)	0.1435
Reproducibility standard deviation (log cfu/g)	0.1622

3.1.9. *LISTERIA MONOCYTOGENES*

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

A significant “effect” of 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 :

Listeria monocytogenes	
Assigned value of the contamination (log cfu/g)	3.241
Assigned value uncertainty (log cfu/g)	0.0081
Standard deviation for proficiency assessment (log cfu/g)	0.0945
Standard deviation for precision (log cfu/g)	0.0696
Interlaboratory’s standard deviation (log cfu/g)	0.0878
Reproducibility standard deviation (log cfu/g)	0.1121

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

3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

Only units n°1, 3, 4 and 5 were artificially contaminated.
265 laboratories obtained correct results.
2 laboratories obtained false positive results (respectively 1 for unit n° 2).
4 laboratories obtained false negative results (respectively 3, 3 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 59th 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.