

PROFICIENCY TEST « RAEMA »



SCHEME N° 77 (11th SEPTEMBER 2023) GENERAL REPORT

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1-GENERAL DATA

1.1. PARTICIPATING LABORATORIES

335 laboratories participated to the 77th scheme. The sending was made on Monday 11th September 2023.

We received **330** answers (98.5%).

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	5	200	53	31	19	1	1	9	6	3	2

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 6.10^2 cfu/g in 5 units ;
- one strain of *Serratia liquefaciens* at a concentration level of 5.10^2 cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of 3.10^2 cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of 4.10^2 cfu/g in 2 units ;
- one strain of *Staphylococcus aureus* at a concentration level of 7.10^3 cfu/g in 5 units ;
- one strain of *Salmonella* Anatum at a concentration level of 50 cfu/g in 1 unit ;
- one strain of *Listeria monocytogenes* at a concentration level of $1,5.10^3$ cfu/g in 3 units.

Samples have been prepared between July and September 2023. The maintenance of bacterial strains, check of their contamination and check of the purity are entrusted to a subcontractor.

1.3.2. SIZE

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

1.3.3. HOMOGENEITY AND STABILITY TEST OF THE CONTAMINATION

Homogeneity and stability of samples are checked during the statistical analysis of participants results. A supplementary check of the contamination's homogeneity was realized on 10 samples for each unit by a double enumeration of aerobic microorganisms at 30°C.

The contamination's stability was also checked by enumeration / detection of all flora on 18, 25 September and 2nd October 2023. These checks were realized by a subcontractor accredited 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

330 laboratories (100%) specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+8	J0+9	J0+10	J0+11	J0+12	J0+14	J0+15
Nb of laboratories	27	53	30	7	4	130	43	8	9	1	1	15	2

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

329 laboratories (99.7%) specified it. The average temperature is **3.9°C** with a standard deviation of 0.9°C. The given data 21, 22, 25, 30 and 30.1°C given by 6 laboratories were not taken into account for this calculation.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF THE SAMPLES

326 laboratories specified it (98.8%).

The average size is **18.4 g** with a standard deviation of 7.9 g. The minimum size indicated is 1 g and the maximum one is 50 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

For 329 answers (99.7%) :

200 laboratories (60.6%) prepare the initial suspension with adding diluent to powder.

125 laboratories (37.9%) prepare the initial suspension with adding powder to diluent.

4 laboratories (1.2%) used another technique to prepare the initial suspension.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

For 328 answers (99.4%) :

291 laboratories (88.2%) use Buffered Peptone Water (or equivalent) for the initial suspension.

34 laboratories (10.3%) use Peptone salt for the initial suspension.

3 laboratories (0.9%) used another diluent for the initial suspension.

2.4. HOMOGENEIZATION TECHNIQUE

For 329 answers (99.7%) :

295 laboratories (89.4%) homogenize their sampling with a StomacherND.

25 laboratories (7.6%) used a manual homogenization.

5 laboratories (1.5%) used a Vortex mixer.

4 laboratories (1.2%) used another technique.

2.5. RESUSCITATION'S CONDITIONS

2.5.1. DURATION

316 laboratories (95.8%) specified it.

The average duration is **27.2 min** with a standard deviation of 16.2 min. The data 1440 min given by one laboratory was not taken into account for this calculation.

2.5.2. TEMPERATURE

316 laboratories (95.8%) specified it.

The average temperature is **21.7°C** with a standard deviation of 3.4°C.

2.6. MICROORGANISM AT 30°C

320 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 4833-1	201
	AFNOR 3M-01/1-09/89	47
	NM ISO 4833-1	25
	AFNOR BIO-12/35-05/13	13
	ISO/NF EN ISO 4833-2	11
	Internal method	9
	XP V08-034	7
	Other	6
	+ Spiral metho	21
Culture medium	Plate Count Agar	234
	Petrifilms	49
	Plate Count Agar + Milk	23
	Tempo AC	13
	Other	1
Preparation	Home made	106
	Ready to use not pre-poured	136
	Ready to use, plate, film, card	75
Plating method	Surface	67
	Pour	234
	Culture medium for card	12
1st dilution retained	- 1	14
	- 2	15
	- 3	220
	- 4	60
	1/400	5
	1/4000	3
	1/40000	1
Incubation temperature	30°C	315
	33-35°C	2
	37°C	2
Incubation duration	68-72.5 h	260
	40-48 h	57
	26 h	1
	120 h	1

2.7. ENTEROBACTERIACEA

283 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-054	109
	→ NM 08.0.109 ⁽¹⁾	15
	ISO/NF EN ISO 21528-2	69
	AFNOR 3M-01/6-09/97	47
	NM ISO 21528-2	15
	AFNOR AES-10/07-01/08	8
	AFNOR BRD-07/24-11/13	8
	AFNOR BIO-12/21-12/06	6
	Internal method	4
	Other	2
Culture medium	VRBG	209
	Petrifilms	50
	Rebecca	9
	Rapid'Enterobacteriaceae	8
	Tempo EB	6
	Other	1
Preparation	Home made	85
	Ready to use not pre-poured	137
	Ready to use, plate, film, card	58
1st dilution retained	- 1	225
	- 2	52
	- 3	1
	1/400	4
Incubation temperature	37°C	186
	30°C	88
	35°C	8
Incubation duration	20-24 h	276
	48 h	6
Confirmatory test	Yes	72
	No	205

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

2.8. TOTAL COLIFORMS

229 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-050	107
	→ <i>NM 08.0.142</i> ⁽²⁾	10
	ISO/NF ISO 4832	53
	NM ISO 4832	21
	AFNOR 3M	17
	AFNOR BIO-12/17-12/05	7
	AFNOR BRD-07/08-12/04	6
	Internal method	4
	Other	4
Culture medium	VRBL	194
	Petrifilms	19
	Rapid Ecoli	7
	Tempo TC	7
	Other	2
Preparation	Home made	83
	Ready to use not pre-poured	119
	Ready to use, plate, film, card	27
1st dilution retained	-1	208
	-2	16
	1/40	1
	1/400	3
Incubation temperature	30°C	217
	35-37°C	12
Incubation duration	20-24 h	226
	48 h	3

AFNOR 3M method including :

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

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

202 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-060	135
	→ NM 08.0.124 ⁽³⁾	29
	AFNOR 3M	22
	ISO/NF ISO 4832	11
	Internal method	3
	Other	2
Culture medium	VRBL	178
	Petrifilms	22
	Other	2
Preparation	Home made	77
	Ready to use not pre-poured	103
	Ready to use, plate, film, card	21
1st dilution retained	-1	192
	-2	8
Incubation temperature	42-45°C	200
	37°C	2
Incubation duration	22-24 h	198
	48 h	3
	30 h	1

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.

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

2.10. ESCHERICHIA COLI

300 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 16649-2	174
	AFNOR 3M	45
	NM ISO 16649-2	25
	AFNOR BRD-07/01-07/93	20
	AFNOR BIO-12/13-02/05	9
	Internal method	7
	AFNOR AES-10/06-01/08	6
	NM 08.0.108	4
	AFNOR BIO-12/05-01/99	3
	ISO/NF EN ISO 16649-3	3
	Other	4
Culture medium	TBX	209
	Petrifilms	45
	Rapid E. coli	23
	Rebecca	8
	Tempo EC	8
	Coli ID	5
	Glutamate + TBX	1
	Other	1
Preparation	Home made	93
	Ready to use not pre-poured	153
	Ready to use, plate, film, card	52
Plating method	Surface	51
	Pour	233
	Culture medium for card	10
1st dilution retained	-1	280
	-2	11
	1/40	1
	1/400	5
Incubation temperature	40-46°C	273
	37°C	25
	30°C	1
Incubation duration	18-26 h	294
	48 h	4
	37 h	1

AFNOR 3M method including :

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

2.11. ANAEROBIC SULFITE-REDUCING BACTERIA

237 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-061	150
	→ NM 08.0.125 ⁽⁴⁾	19
	ISO/NF ISO 15213-1	38
	NM ISO 15213	14
	Internal method	9
	Other	6
Culture medium	TSC	221
	TSN	8
	Iron Sulfite agar	7
	Other	1
Preparation	Home made	83
	Ready to use not pre-poured	124
	Ready to use, plate, film, card	29
Seeding way	Plates	161
	Tubes	72
1st dilution retained	-1	180
	-2	55
Incubation temperature	44-47°C	177
	37°C	60
Incubation duration	16-24 h	200
	48 h	31
	72 h	6

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

2.12. CLOSTRIDIUM PERFRINGENS

194 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 7937	156
	NM ISO 7937	27
	NM 08.0.111	2
	Internal method	2
	Other	6
Culture medium	TSC	193
Preparation	Home made	63
	Ready to use not pre-poured	125
	Ready to use, plate, film, card	5
1st dilution retained	-1	171
	-2	22
Incubation temperature	37°C	188
	44-46°C	5
Incubation duration	18-24 h	187
	48 h	6
Confirmation test	None	37
	Lactose-sulfite	141
	Strip	5
	MALDI-TOF mass spectrometry	3
	Other	2

2.13. COAGULASE POSITIVE STAPHYLOCOCCI

298 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 6888-2	134
	ISO/NF EN ISO 6888-1	75
	AFNOR BKR-23/10-12/15	24
	NM ISO 6888-1	17
	AFNOR BIO-12/28-04/10	13
	AFNOR 3M-01/9-04/03	11
	Internal method	7
	NM ISO 6888-2	6
	ISO/NF EN ISO 6888-3	3
	NM 08.0.112	2
	NordVal No :049	2
	Other	4
Culture medium	RPF	131
	BP+egg yolk tellurite	98
	Easy Staph	27
	Petrifilm	13
	Tempo STA	13
	BP+egg yolk tellurite+ sulfamethazine	11
	Rapid Staph	2
	Other	3
Preparation	Home made	74
	Ready to use not pre-poured	127
	Ready to use, plate, film, cards	93
Plating method	Surface	148
	Pour	135
	Culture medium for card	13
1st dilution retained	-1	105
	-2	171
	-3	12
	1/40	3
	1/400	5
Incubation temperature	35-37°C	291
	27-34°C	4
	44-48°C	2
Incubation duration	42-48 h	200
	20-26 h	96
	30 h	1
Confirmation test	None	186
	Staphylo-coagulase	79
	Clumping factor	12
	DNase	6
	MALDI-TOF mass spectrometry	3
	Other	4

2.14. LISTERIA MONOCYTOGENES – ENUMERATION

233 laboratories performed the enumeration.

RESUSCITATION

78 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	62
	ISO/NF EN ISO 11290-2	59
	AFNOR BKR-23/05-12/07	56
	AFNOR BRD-07/05-09/01	26
	NM ISO 11290-2	19
	AFNOR BRD-07/17-01/09	9
	Other	2
Resuscitation medium	Buffered Peptone Water or equivalent	194
	Half-fraser	33
	Fraser base	1
	Other	3
Enumeration medium	ALOA Count	106
	Compass Listeria	80
	Rapid Lmono	27
	AL Agar	12
	OCLA	2
	Palcam	2
	Other	1
Preparation	Home made	37
	Ready to use not pre-poured	47
	Ready to use, plate, film, card	145
Plating method	Surface	189
	Pour	41

Parameters	Mode	Nb laboratories
1st dilution retained	-1	190
	-2	41
Incubation temperature	37°C	229
	30°C	2
	41.5°C	1
Incubation duration	44-48.5 h	191
	20-24 h	39
	34-40 h	2
Confirmation test	None	43
	Biochemical	134
	Biochemical + CAMP	36
	MALDI-TOF mass spectrometry	4
	Other	5
Nb of colonies tested per plate	1	65
	2-4	12
	5	97
	6	1
	150	1
	300	1

2.15. SALMONELLA – DETECTION

297 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	83
	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	71
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	32
	NM ISO 6579-1	31
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	26
	AFNOR BIO 12/41-03/17 (SALMA One day)	20
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	13
	AFNOR BIO 12/01-04/94 (VIDAS SLM)	7
	AFNOR UNI 03/07-11/13 (PCR)	3
	AFNOR UNI 03/06-12/07 (Salmonella precis)	3
	AFNOR BIO 12/38-06/16 (GENE UP Salmonella)	2
	AFNOR BRD 07/06-07/04 (PCR)	2
	Internal method	1
	Other	3

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 114 laboratories using ISO/NF EN ISO 6579-1 and NM ISO 6579-1 method, 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 6579-1	83
	NM ISO 6579-1	31
	Internal method	1
	Other	3
Pre-enrichment medium	None pre-enrichment	1
	Buffered Peptone Water	115
	Other	1
Pre-enrichment temperature	37±1°C	115
	41.5°C	1
	22°C	1
Pre-enrichment duration	16-20 h	89
	22-24 h	28
Enrichment medium	None enrichment	1
	RVS	112
	MKTTn	109
	Selenite-cystine broth	32
	Other	2
Isolation medium	XLD	106
	Hektoen	35
	Bismuth Sulfate	27
	IRIS Salmonella agar	14
	ASAP	13
	GVB	11
	SS	11
	Rapid Salmonella	8
	Compass Salmonella	3
	Rambach	1
	Brilliance Salmonella	1
	Other	7
Confirmation test	Biochemical	40
	Biochemical + serological agglutination	68
	MALDI-TOF mass spectrometry	6
	Other	1

2.16. LISTERIA MONOCYTOGENES – DETECTION

271 laboratories performed the detection.

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	60
	AFNOR BKR 23/02-11/02 (Compass L. mono)	59
	AFNOR AES 10/03-09/00 (ALOA one day)	58
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	26
	NM ISO 11290-1	21
	AFNOR BRD 07/16-01/09 (Agar Listeria)	9
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	7
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	7
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	6
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	4
	AFNOR BRD 07/10-04/05 (IQ Check Listeria)	4
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	3
	AFNOR UNI 03/08-11/13 (PCR)	2
	AFNOR UNI 03/04-04/05 (Listeria Precis)	2
	Internal method	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 12/40-11/16 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 81 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 methods, and the 3 laboratories using an internal or another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	60
	NM ISO 11290-1	21
	Internal method	1
	Other	2
Primary enrichment medium	None primary enrichment	1
	Half-Fraser	78
	One broth Listeria	1
	Other	2
Primary enrichment temperature	30°C	73
	37°C	7
	20°C	1
Primary enrichment duration	22-27 h	80
	48 h	1
Secondary enrichment medium	None secondary enrichment	6
	Fraser	74
	Other	1
Secondary enrichment temperature	37°C	72
	30°C	4
Secondary enrichment duration	22-24 h	61
	48 h	15
Isolation medium	Palcam	58
	Ottaviani et Agosti	46
	Compass Listeria	29
	Oxford	13
	Rapid L'mono	3
	Other	2
Isolation temperature	37±1°C	80
	30°C	1
Isolation duration	48 h	49
	24-26 h	32
Confirmation test	None	4
	Biochemical	47
	Biochemical + CAMP	27
	MALDI-TOF mass spectrometry	3
Nb of colonies per plate	1	29
	2-3	7
	5	34
	12	1
	300	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 used to assess the trueness and the reference standard deviation for the assessment of the precision are consensual values obtained with the results of all the participants. These values are obtained by robust estimation methods in order to eliminate influence of aberrant results. However some results are excluded of the statistical analysis. That is the case when laboratories do not give results for all contaminated units, when results are “less than CFU/g”, when samples are analyzed after the deadline (time of receipt > 4 days after sending or time of analysis > 15 days after sending) or when this information is not specified.

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

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

PRECISION

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

The standard deviation of your results, s , is compared to the robust estimation of the standard deviation (reference standard deviation of precision), s^* , obtained with algorithm S from the standard 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 characterized by its own assigned value.

The assigned value uncertainty is calculated with the following formula :

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

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,
- $2,0 < |z| < 3,0$ 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 laboratories' 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)	5.372
Assigned value uncertainty (log cfu/g)	0.0066
Standard deviation for proficiency assessment (log cfu/g)	0.0912
Standard deviation for precision (log cfu/g)	0.0540
Interlaboratory's standard deviation (log cfu/g)	0.0880
Reproducibility standard deviation (log cfu/g)	0.1032

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)	2.720	3.074
Assigned value uncertainty (log cfu/g)	0.0197	0.0292
Standard deviation for proficiency assessment (log cfu/g)	0.2271	0.1730
Standard deviation for precision (log cfu/g)	0.1094	
Interlaboratory's standard deviation (log cfu/g)	0.2217	0.1660
Reproducibility standard deviation (log cfu/g)	0.2472	0.1988

3.1.3. TOTAL COLIFORMS

A significant "effect" of the culture medium, 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	Group 2	Group 3
Assigned value of the contamination (log cfu/g)	2.629	2.775	3.136
Assigned value uncertainty (log cfu/g)	0.0253	0.0388	0.0601
Standard deviation for proficiency assessment (log cfu/g)	0.2467	0.2058	0.1984
Standard deviation for precision (log cfu/g)	0.1106		
Interlaboratory's standard deviation (log cfu/g)	0.2416	0.1997	0.1921
Reproducibility standard deviation (log cfu/g)	0.2652	0.2277	0.2211

Comment : Due to the low number of laboratories included in group 3, the assigned value uncertainty is not insignificant (cf NF ISO 13528 §9.2.1). Laboratories included in the group 3 obtain a satisfactory z-score (without impact), except two laboratories with an advertisement signal and one laboratory with an action signal. These three laboratories have been warned.

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)	2.562
Assigned value uncertainty (log cfu/g)	0.0203
Standard deviation for proficiency assessment (log cfu/g)	0.2205
Standard deviation for precision (log cfu/g)	0.1291
Interlaboratory's standard deviation (log cfu/g)	0.2128
Reproducibility standard deviation (log cfu/g)	0.2393

3.1.5. ESCHERICHIA COLI

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 :

<i>Escherichia coli</i>	
Assigned value of the contamination (log cfu/g)	2.478
Assigned value uncertainty (log cfu/g)	0.0133
Standard deviation for proficiency assessment (log cfu/g)	0.1763
Standard deviation for precision (log cfu/g)	0.1334
Interlaboratory's standard deviation (log cfu/g)	0.1659
Reproducibility standard deviation (log cfu/g)	0.2129

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°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 :

Anaerobic sulfite-reducing bacteria	
Assigned value of the contamination (log cfu/g)	2.585
Assigned value uncertainty (log cfu/g)	0.0165
Standard deviation for proficiency assessment (log cfu/g)	0.1924
Standard deviation for precision (log cfu/g)	0.1008
Interlaboratory's standard deviation (log cfu/g)	0.1787
Reproducibility standard deviation (log cfu/g)	0.2051

Comment :

- 13 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 20 to 2700 cfu/g.
- 10 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 20 to 1200 cfu/g.
- 10 laboratories detected ASR in unit n°3 non-artificially contaminated by *C. perfringens* with a contamination level from 15 to 1000 cfu/g.

3.1.7. *CLOSTRIDIUM PERFRINGENS*

Only units n°4 and 5 were artificially contaminated.

A significant “effect” of the homogeneization technique 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 :

<i>Clostridium perfringens</i>	
Assigned value of the contamination (log cfu/g)	2.582
Assigned value uncertainty (log cfu/g)	0.0185
Standard deviation for proficiency assessment (log cfu/g)	0.1960
Standard deviation for precision (log cfu/g)	0.0960
Interlaboratory's standard deviation (log cfu/g)	0.1838
Reproducibility standard deviation (log cfu/g)	0.2074

Comment :

- 3 laboratories detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 360 to 500 cfu/g.
- 2 laboratories detected *C. perfringens* in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level of 300 cfu/g.
- 3 laboratories detected *C. perfringens* in unit n°3 non-artificially contaminated by *C. perfringens* with a contamination level of 30 to 600 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)	3.888
Assigned value uncertainty (log cfu/g)	0.0112
Standard deviation for proficiency assessment (log cfu/g)	0.1493
Standard deviation for precision (log cfu/g)	0.0653
Interlaboratory's standard deviation (log cfu/g)	0.1464
Reproducibility standard deviation (log cfu/g)	0.1603

3.1.9. *LISTERIA MONOCYTOGENES*

Only units n°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 :

<i>Listeria monocytogenes</i>	
Assigned value of the contamination (log cfu/g)	3.175
Assigned value uncertainty (log cfu/g)	0.0109
Standard deviation for proficiency assessment (log cfu/g)	0.1281
Standard deviation for precision (log cfu/g)	0.0754
Interlaboratory's standard deviation (log cfu/g)	0.1205
Reproducibility standard deviation (log cfu/g)	0.1421

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°5 was artificially contaminated.

281 laboratories obtained correct results.

12 laboratories obtained false positive results (respectively 3, 3, 6 and 4 false-positive for units n° 1, 2, 3 and 4).

9 laboratories obtained false negative results (respectively 9 false-negative for units n° 5).

3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

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

263 laboratories obtained correct results.

6 laboratories obtained false positive results (respectively 5 and 2 false-positive for units n° 1 and 2).

7 laboratories obtained false negative results (respectively 3, 1 and 4 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 57th 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.