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PROFICIENCY TEST « RAEMA »

SCHEME N° 76
(6th MARCH 2023)
GENERAL REPORT

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

[1] Coordinator of the proficiency test « RAEMA »

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1- GENERAL DATA

1.1. PARTICIPATING LABORATORIES

336 laboratories participated to the 76th scheme. The sending was made on Monday 6th March 2023. We received **332** answers (98.8%).

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+11	J0+13	J0+15	J0+16
Nb of laboratories	3	222	60	21	8	3	6	3	2	1	2	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

The sample included :
- one strain of *Enterococcus sp*. At a concentration level of $1{,}5.10^5$ 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 3 units ;
- one strain of *Staphylococcus aureus* at a concentration level of $4{,}5.10^3$ 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 8.10^2 cfu/g in 2 units.

Samples have been prepared between January and March 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 13, 20 and 27 March 2023. These checks were realized by a subcontractor accreditated by Cofrac.
Homogeneity of samples has been validated. Stability of samples has been validated, except for "coagulase positive staphylococci" in the 3rd week of analysis. Only laboratories having analyzed this parameter from J14 are concerned and informed.

1.3.4. FLORA FOR ENUMERATION / DETECTION

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

1.4. EXECUTION OF ANALYZES

1.4.1. DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

332 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+12	J0+14	J0+15	J0+16	J0+17
Nb of laboratories	27	59	27	9	1	130	45	14	5	1	8	3	2	1

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

332 laboratories (100%) specified it. The average temperature is **3.8°C** with a standard deviation of 0.9°C. The given data 15, 19, 20, 22 and 25°C given by 5 laboratories were not taken into account for this calculation.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF THE SAMPLES

326 laboratories specified it (98.2%).
The average size is **17.7 g** with a standard deviation of 7.9 g. The minimum size indicated is 1 g and the maximum one is 50 g. The given data 0.1 and 340g given by 2 laboratories were not taken into account for this calculation.

2.2. PREPARATION OF THE INITIAL SUSPENSION

For **332** answers (100%) :
196 laboratories (59.0%) prepare the initial suspension with adding diluent to powder.
133 laboratories (40.1%) prepare the initial suspension with adding powder to diluent.
3 laboratories (0.9%) used another technique to prepare the initial suspension.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

For **330** answers (99.4%) :
290 laboratories (87.4%) use Buffered Peptone Water (or equivalent) for the initial suspension.
37 laboratories (11.1%) use Peptone salt for the initial suspension.
3 laboratories (0.9%) used another diluent for the initial suspension.

2.4. HOMOGENEIZATION TECHNIQUE

For **327** answers (98.5%) :
298 laboratories (89.8%) homogeneize their sampling with a StomacherND.
23 laboratories (6.9%) used a manual homogenization.
2 laboratories (0.6%) used a Vortex mixer.
4 laboratories (1.2%) used another technique.

2.5. RESUSCITATION'S CONDITIONS

2.5.1. DURATION

316 laboratories (95.2%) specified it.
The average duration is **27.3 min** with a standard deviation of 16.0 min. The data 120 and 1440 min given by 4 laboratories were not taken into account for this calculation.

2.5.2. TEMPERATURE

314 laboratories (94.6%) specified it.
The average temperature is **21.3°C** with a standard deviation of 3.3°C.

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2.6. MICROORGANISM AT 30°C

323 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 4833-1	206
	AFNOR 3M-01/1-09/89	45
	NM ISO 4833-1	27
	ISO/NF EN ISO 4833-2	12
	AFNOR BIO-12/35-05/13	11
	Internal method	8
	XP V08-034	6
	Other	8
	+ V08-100 (spiral)	14
Culture medium	Plate Count Agar	240
	Petrifilms	46
	Plate Count Agar + Milk	25
	Tempo AC	11
	Other	1
Preparation	Home made	111
	Ready to use not pre-poured	145
	Ready to use, plate, film, card	67
Plating method	Surface	61
	Pour	244
	Culture medium for card	11
1st dilution retained	- 1	7
	- 2	13
	- 3	265
	- 4	28
	1/400	9
Incubation temperature	30°C	315
	32-35°C	4
	37°C	2
	47°C	1
Incubation duration	64-76 h	265
	42-48 h	53
	24-26 h	3
	144 h	1
	Other	1

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2.7. ENTEROBACTERIACEA

283 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-054	106
	→ *NM 08.0.109*(1)	14
	ISO/NF EN ISO 21528-2	73
	AFNOR 3M-01/6-09/97	44
	NM ISO 21528-2	17
	AFNOR BIO-12/21-12/06	10
	AFNOR AES-10/07-01/08	8
	AFNOR BRD-07/24-11/13	7
	Internal method	2
	Other	2
Culture medium	VRBG	208
	Petrifilms	47
	Tempo EB	10
	Rebecca	9
	Rapid'Enterobacteriaceae	8
Preparation	Home made	88
	Ready to use not pre-poured	134
	Ready to use, plate, film, card	59
1st dilution retained	- 1	226
	- 2	49
	1/40	2
	1/400	6
Incubation temperature	37±1°C	180
	30°C	92
	35°C	10
Incubation duration	20-25 h	275
	44-48 h	6
	Other	1
Confirmatory test	Yes	77
	No	202

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

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2.8. TOTAL COLIFORMS

231 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-050	109
	→ *NM 08.0.142*(2)	11
	ISO/NF ISO 4832	59
	NM ISO 4832	23
	AFNOR 3M	14
	AFNOR BRD-07/08-12/04	5
	AFNOR BIO-12/17-12/05	4
	Internal method	2
	Other	4
Culture medium	VRBL	202
	Petrifilms	16
	Rapid Ecoli	6
	Tempo TC	4
	Other	3
Preparation	Home made	88
	Ready to use not pre-poured	124
	Ready to use, plate, film, card	19
1st dilution retained	-1	211
	-2	17
	1/400	3
Incubation temperature	30-32°C	213
	37°C	16
	44°C	1
Incubation duration	20-26 h	225
	48 h	4
	Other	1

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.*

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2.9. THERMOTOLERANT COLIFORMS

207 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-060	139
	→ *NM 08.0.124*(3)	31
	AFNOR 3M	21
	ISO/NF ISO 4832	11
	Internal method	2
	Other	3
Culture medium	VRBL	182
	Petrifilms	22
	Other	3
Preparation	Home made	83
	Ready to use not pre-poured	104
	Ready to use, plate, film, card	20
1st dilution retained	-1	191
	-2	16
Incubation temperature	42-45°C	205
	37°C	2
Incubation duration	22-25 h	200
	48 h	4
	27-30 h	2
	Other	1

AFNOR 3M method including :

3 laboratories specified utilization of AFNOR 3M-01/02-09/89 C method.

1 laboratory specified utilization of AFNOR 3M-01/05-03/97 B method.

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

2.10. ESCHERICHIA COLI

305 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF ISO 16649-2	178
	AFNOR 3M	43
	NM ISO 16649-2	27
	AFNOR BRD-07/01-07/93	19
	AFNOR BIO-12/13-02/05	9
	AFNOR AES-10/06-01/08	8
	NM 08.0.108	5
	AFNOR BIO-12/05-01/99	4
	ISO/NF EN ISO 16649-3	4
	Internal method	2
	Other	6
Culture medium	TBX	214
	Petrifilms	44
	Rapid E. coli	21
	Rebecca	10
	Tempo EC	9
	Coli ID	6
	Glutamate + TBX	1
Preparation	Home made	94
	Ready to use not pre-poured	158
	Ready to use, plate, film, card	51
Plating method	Surface	45
	Pour	246
	Culture medium for card	9
1st dilution retained	-1	282
	-2	15
	1/40	1
	1/400	6
Incubation temperature	41-46°C	271
	37±1°C	31
	30°C	2
Incubation duration	18-27 h	298
	48 h	5
	Other	1

AFNOR 3M method including :

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

2.11. ANAEROBIC SULFITE-REDUCING BACTERIA

243 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	NF V08-061	156
	→ *NM 08.0.125*[4]	19
	ISO/NF ISO 15213	40
	NM ISO 15213	14
	Internal method	7
	Other	7
Culture medium	TSC	230
	TSN	7
	Iron Sulfite agar	5
	Other	1
Preparation	Home made	87
	Ready to use not pre-poured	129
	Ready to use, plate, film, card	27
Seeding way	Plates	162
	Tubes	80
1st dilution retained	-1	181
	-2	61
Incubation temperature	44-47°C	178
	37°C	64
Incubation duration	16-25 h	202
	44-48 h	33
	72 h	5
	9 h	1
	Other	1

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

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2.12. CLOSTRIDIUM PERFRINGENS

192 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 7937	151
	NM ISO 7937	32
	NM 08.0.111	2
	Internal method	1
	Other	6
Culture medium	TSC	190
	Other	2
Preparation	Home made	58
	Ready to use not pre-poured	127
	Ready to use, plate, film, card	6
1st dilution retained	-1	168
	-2	23
Incubation temperature	37°C	182
	44-46°C	9
	32°C	1
Incubation duration	18-25 h	184
	44-48 h	7
	72 h	1
Confirmation test	None	31
	Lactose-sulfite	141
	Strip	8
	MALDI-TOF mass spectrometry	5

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2.13. COAGULASE POSITIVE STAPHYLOCOCCI

302 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
Method	ISO/NF EN ISO 6888-2	136
	ISO/NF EN ISO 6888-1	71
	NM ISO 6888-1	27
	AFNOR BKR-23/10-12/15	23
	AFNOR 3M-01/9-04/03	12
	AFNOR BIO-12/28-04/10	10
	NM ISO 6888-2	7
	Internal method	4
	NM 08.0.112	2
	NordVal No :049	2
	ISO/NF EN ISO 6888-3	2
	Other	5
Culture medium	RPF	129
	BP+egg yolk tellurite	96
	Easy Staph	30
	BP+egg yolk tellurite+ sulfamethazine	17
	Petrifilm	13
	Tempo STA	10
	Rapid Staph	2
	Other	3
Preparation	Home made	77
	Ready to use not pre-poured	129
	Ready to use, plate, film, cards	93
Plating method	Surface	150
	Pour	139
	Culture medium for card	10
1st dilution retained	-1	123
	-2	169
	-3	1
	1/40	5
	1/400	3
Incubation temperature	37±1°C	299
	30-32°C	2
Incubation duration	42-49 h	208
	21-25 h	89
	72 h	1
	34 h	1
	52 h	1
	Other	1
Confirmation test	None	188
	Staphylo-coagulase	87
	Clumping factor	11
	DNase	7
	MALDI-TOF mass spectrometry	3
	Other	3

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2.14. LISTERIA MONOCYTOGENES – ENUMERATION

237 laboratories performed the enumeration.

RESUSCITATION

76 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	69
	AFNOR AES-10/05-09/06	55
	AFNOR BKR-23/05-12/07	52
	NM ISO 11290-2	25
	AFNOR BRD-07/05-09/01	21
	AFNOR BRD-07/17-01/09	12
	Internal method	1
	Other	2
Resuscitation medium	Buffered Peptone Water or equivalent	189
	Half-fraser	45
	Fraser base	1
	Other	2
Enumeration medium	ALOA Count	110
	Compass Listeria	81
	Rapid Lmono	22
	AL Agar	18
	OCLA	2
	Palcam	2
	Other	2
Preparation	Home made	42
	Ready to use not pre-poured	46
	Ready to use, plate, film, card	148
Plating method	Surface	196
	Pour	40
	Culture medium for card	1

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Parameters	Mode	Nb laboratories
1st dilution retained	-1	223
	-2	13
Incubation temperature	37±1°C	233
	30-32°C	4
Incubation duration	44-48.5 h	200
	24-25 h	36
	4 h	1
Confirmation test	None	41
	Biochemical	145
	Biochemical + CAMP	35
	MALDI-TOF mass spectrometry	6
	Other	3
Nb of colonies tested per plate	1	62
	2-4	14
	5	106
	6	2
	10	1
	48	1
	150	1

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2.15. SALMONELLA – DETECTION

301 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)	76
	ISO/NF EN ISO 6579-1	74
	NM ISO 6579-1	37
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	29
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	23
	AFNOR BIO 12/41-03/17 (SALMA One day)	19
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	13
	AFNOR BIO 12/01-04/94 (VIDAS SLM)	9
	AFNOR UNI 03/07-11/13 (PCR)	4
	AFNOR UNI 03/06-12/07 (Salmonella precis)	4
	AFNOR BIO 12/38-06/16 (GENE UP Salmonella)	3
	AFNOR BRD 07/06-07/04 (PCR)	2
	AFNOR TRA 02/08-03/01 (TRANSIA PLATE Salmonella GOLD)	1
	Internal method	1
	Other	6

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 111 laboratories using ISO/NF EN ISO 6579-1 and NM ISO 6579-1 method, 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 6579-1	74
	NM ISO 6579-1	37
	Internal method	1
	Other	6
Pre-enrichment medium	Buffered Peptone Water	117
	Other	1
Pre-enrichment temperature	37±1°C	114
	41.5-42.5°C	2
	22°C	1
	32°C	1
Pre-enrichment duration	16-21 h	83
	22-24 h	35
Enrichment medium	None enrichment	3
	RVS	110
	MKTTn	106
	Selenite-cystine broth	29
	Other	1
Isolation medium	XLD	106
	Hektoen	31
	Bismuth Sulfate	26
	GVB	17
	ASAP	14
	SS	11
	IRIS Salmonella agar	10
	Rapid Salmonella	8
	Compass Salmonella	5
	Rambach	3
	Brilliance Salmonella	2
	Other	5
Confirmation test	Biochemical	41
	Biochemical + serological agglutination	68
	MALDI-TOF mass spectrometry	4
	Other	1

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2.16. LISTERIA MONOCYTOGENES – DETECTION

275 laboratories performed the detection.

Parameter	Mode	Nb laboratories
Method	ISO/NF EN ISO 11290-1	56
	AFNOR BKR 23/02-11/02 (Compass L. mono)	54
	AFNOR AES 10/03-09/00 (ALOA one day)	52
	NM ISO 11290-1	33
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	20
	AFNOR BRD 07/16-01/09 (Agar Listeria)	14
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	11
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	6
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	5
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	4
	AFNOR UNI 03/08-11/13 (PCR)	4
	AFNOR UNI 03/04-04/05 (Listeria Precis)	3
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	3
	AFNOR BRD 07/10-04/05 (IQ Check Listeria)	3
	Internal method	2
	Other	5

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

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The detail of the methodology followed by 89 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	56
	NM ISO 11290-1	33
	Internal method	2
	Other	5
Primary enrichment medium	None primary enrichment	1
	Half-Fraser	87
	One broth Listeria	2
	Other	6
Primary enrichment temperature	30°C	88
	37°C	7
	32°C	1
Primary enrichment duration	22-28 h	95
	48 h	1
Secondary enrichment medium	None secondary enrichment	8
	Fraser	86
	Other	1
Secondary enrichment temperature	37±1°C	85
	30°C	2
	32°C	1
Secondary enrichment duration	22-24 h	73
	48 h	15
Isolation medium	Palcam	66
	Ottaviani et Agosti	50
	Compass Listeria	35
	Oxford	16
	Rapid L'mono	7
	Brilliance Listeria	2
Isolation temperature	37±1°C	92
	30-32°C	4
Isolation duration	44-48.5 h	57
	24 h	39
Confirmation test	None	6
	Biochemical	59
	Biochemical + CAMP	27
	MALDI-TOF mass spectrometry	3
Nb of colonies per plate	1	27
	2-4	8
	5	41
	10	1
	25	1

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3. ASSESSMENT OF PERFORMANCE (INDIVIDUEL REPORTS)

3.1. PERFORMANCES IN ENUMERATION

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

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

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

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

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

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

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

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

TRUENESS

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

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

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

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

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

INDIVIDUAL REPORTS – FOR EACH CRITERIA YOU FIND THE FOLLOWING INFORMATIONS

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

3.1.1. MICROORGANISMS AT 30°C

None significant effect of the analysis technique has been highlighted.

Microorganisms at 30°C	
Assigned value of the contamination (log cfu/g)	5.242
Assigned value uncertainty (log cfu/g)	0.0061
Standard deviation for proficiency assessment (log cfu/g)	0.0847
Standard deviation for precision (log cfu/g)	0.0476
Interlaboratory's standard deviation (log cfu/g)	0.0820
Reproducibility standard deviation (log cfu/g)	0.0948

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 two groups :

Enterobacteriaceae	Group 1	Group 2
Assigned value of the contamination (log cfu/g)	2.729	3.067
Assigned value uncertainty (log cfu/g)	0.0190	0.0259
Standard deviation for proficiency assessment (log cfu/g)	0.2140	0.1735
Standard deviation for precision (log cfu/g)	0.1050	
Interlaboratory's standard deviation (log cfu/g)	0.2088	0.1671
Reproducibility standard deviation (log cfu/g)	0.2337	0.1973

3.1.3. TOTAL COLIFORMS

A significant "effect" of the culture medium, 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)	2.696
Assigned value uncertainty (log cfu/g)	0.0201
Standard deviation for proficiency assessment (log cfu/g)	0.2362
Standard deviation for precision (log cfu/g)	0.0970
Interlaboratory's standard deviation (log cfu/g)	0.2322
Reproducibility standard deviation (log cfu/g)	0.2548

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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)	2.689
Assigned value uncertainty (log cfu/g)	0.0195
Standard deviation for proficiency assessment (log cfu/g)	0.2176
Standard deviation for precision (log cfu/g)	0.0987
Interlaboratory's standard deviation (log cfu/g)	0.2130
Reproducibility standard deviation (log cfu/g)	0.2375

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 :

Escherichia coli	
Assigned value of the contamination (log cfu/g)	2.603
Assigned value uncertainty (log cfu/g)	0.0127
Standard deviation for proficiency assessment (log cfu/g)	0.1724
Standard deviation for precision (log cfu/g)	0.1085
Interlaboratory's standard deviation (log cfu/g)	0.1654
Reproducibility standard deviation (log cfu/g)	0.1978

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

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

A significant "effect" of the homogeneization technique, 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)	2.622
Assigned value uncertainty (log cfu/g)	0.0154
Standard deviation for proficiency assessment (log cfu/g)	0.1854
Standard deviation for precision (log cfu/g)	0.0949
Interlaboratory's standard deviation (log cfu/g)	0.1772
Reproducibility standard deviation (log cfu/g)	0.2010

Comment :
- 4 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 1 to 380 cfu/g.
- 4 laboratories detected ASR in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 1 to 800 cfu/g.

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3.1.7. *CLOSTRIDIUM PERFRINGENS*

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

A significant "effect" of the preparation mode of the culture medium, the confirmation test 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)	2.625
Assigned value uncertainty (log cfu/g)	0.0161
Standard deviation for proficiency assessment (log cfu/g)	0.1745
Standard deviation for precision (log cfu/g)	0.0833
Interlaboratory's standard deviation (log cfu/g)	0.1678
Reproducibility standard deviation (log cfu/g)	0.1873

Comment :
- 2 laboratories detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 1 to 380 cfu/g.
- 1 laboratory detected *C. perfringens* in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level of 1 cfu/g.

3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

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

Coagulase positive Staphylococci	
Assigned value of the contamination (log cfu/g)	3.696
Assigned value uncertainty (log cfu/g)	0.0123
Standard deviation for proficiency assessment (log cfu/g)	0.1650
Standard deviation for precision (log cfu/g)	0.0732
Interlaboratory's standard deviation (log cfu/g)	0.1618
Reproducibility standard deviation (log cfu/g)	0.1776

3.1.9. *LISTERIA MONOCYTOGENES*

Only units n°2 and 3 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

Listeria monocytogenes	
Assigned value of the contamination (log cfu/g)	2.923
Assigned value uncertainty (log cfu/g)	0.0097
Standard deviation for proficiency assessment (log cfu/g)	0.1160
Standard deviation for precision (log cfu/g)	0.0642
Interlaboratory's standard deviation (log cfu/g)	0.1060
Reproducibility standard deviation (log cfu/g)	0.1246

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

3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

Only units n°2 and 3 were artificially contaminated.
274 laboratories obtained correct results.
No laboratory obtained false positive results.
1 laboratory obtained false negative results (respectively 1 and 1 false-negative for units n°2 and 3).

3.3. EVOLUTION OF PERFORMANCE

You will find, on each page of your performance's assessment, a graph representing evolution of it on different tests since the 56th scheme.

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

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

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