

## PROFICIENCY TEST « RAEMA »



### SCHEME N° 73 (4th OCTOBER 2021) GENERAL REPORT

« Any reproduction of the report must be made in its entirety »  
« The Cofrac logo may not be used outside this report »

**V. CARLIER<sup>(1)</sup>, L. ALI-MANDJEE et M. CARLIER**  
ASA - ENVA, 7 avenue du Général de Gaulle, 94704 MAISONS ALFORT CEDEX

<sup>(1)</sup> Coordinator of the proficiency test « RAEMA »

## Table of contents

<b>1- GENERAL DATA</b> .....	<b>3</b>
1-1 PARTICIPATING LABORATORIES .....	3
1-2 DELIVERY TIME OF THE PARCEL .....	3
1-3 INFORMATION ABOUT SAMPLE .....	3
1-3-1 NATURE .....	3
1-3-2 SIZE .....	3
1-3-3 HOMOGENEITY AND STABILITY OF THE CONTAMINATION.....	3
1-3-4 FLORA FOR ENUMERATION / DETECTION.....	3
1-4 EXECUTION OF ANALYSIS .....	4
1-4-1 DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES .....	4
1-4-2 PRESERVATION TEMPERATURE OF SAMPLE BEFORE ANALYSIS .....	4
<b>2- EXPLOITATION OF ANALYSIS REPORT</b> .....	<b>4</b>
2-1 PREPARATION OF THE INITIAL SUSPENSION.....	4
2-2 DILUENT USED FOR THE INITIAL SUSPENSION .....	4
2-3 HOMOGENEIZATION TECHNIQUE .....	4
2-4 RESUSCITATION'S CONDITIONS .....	4
2-4-1 DURATION .....	4
2-4-2 TEMPERATURE.....	4
2-5 MICROORGANISMS AT 30°C .....	5
2-6 ENTEROBACTERIACEA .....	6
2-7 TOTAL COLIFORMS .....	7
2-8 THERMOTOLERANT COLIFORMS.....	8
2-9 ESCHERICHIA COLI.....	9
2-10 ANAEROBIC SULFITE-REDUCING BACTERIA.....	10
2-11 CLOSTRIDIUM PERFRINGENS .....	11
2-12 COAGULASE POSITIVE STAPHYLOCOCCI .....	12
2-13 LISTERIA MONOCYTOGENES – ENUMERATION .....	13
2-14 SALMONELLA –DETECTION.....	15
2-15 LISTERIA MONOCYTOGENES –DETECTION .....	17
<b>3- ASSESSMENT OF PERFORMANCE</b> .....	<b>19</b>
3-1 PERFORMANCES IN ENUMERATION.....	19
3-1-1 MICROORGANISMS AT 30°C .....	21
3-1-2 ENTEROBACTERIACEA .....	21
3-1-3 TOTAL COLIFORMS.....	21
3-1-4 THERMOTOLERANT COLIFORMS .....	22
3-1-5 ESCHERICHIA COLI .....	22
3-1-6 ANAEROBIC SULFITE-REDUCING BACTERIA.....	22
3-1-7 CLOSTRIDIUM PERFRINGENS .....	23
3-1-8 COAGULASE POSITIVE STAPHYLOCOCCI.....	23
3-1-9 LISTERIA MONOCYTOGENES – ENUMERATION .....	23
3-2 PERFORMANCES IN DETECTION.....	24
3-2-1 DETECTION – SALMONELLA .....	24
3-2-2 DETECTION - LISTERIA MONOCYTOGENES.....	24
3-3 EVOLUTION OF PERFORMANCE .....	24

## 1-GENERAL DATA

### 1.1.PARTICIPATING LABORATORIES

**345 laboratories** participated to the 73<sup>th</sup> scheme. The sending was made on Monday 4th October 2021. We received **338** answers (98.0%).

### 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+11	J0+18
Nb of laboratories	6	235	34	21	16	4	1	7	5	7	1	1

### 1.3.INFORMATIONS ABOUT SAMPLE

#### 1.3.1. NATURE

The sample included :

- one strain of *Enterococcus sp.* at a concentration level of  $10^5$  cfu/g in 5 units ;
- one strain of *Citrobacter sp.* at a concentration level of  $5.10^2$  cfu/g in 5 units ;
- one strain of *Serratia liquefaciens* at a concentration level of  $5.10^2$  cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of  $1,5.10^2$  cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of  $5.10^2$  cfu/g in 3 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 25 cfu/g in 2 units ;
- one strain of *Listeria monocytogenes* at a concentration level of  $2.10^3$  cfu/g in 3 units .

#### 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 11, 18 and 25 October 2021. 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

338 laboratories (100%) specified it.

Analysis time	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+11	J0+14	J0+15	J0+18	J0+21
Nb of laboratories	1	41	42	32	4	1	2	135	46	14	7	3	5	3	1	1

### 1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

333 laboratories (98.5%) specified it. The average temperature is **3.9°C** with a standard deviation of 0.9°C. The given data 19, 20, 21, 22, 25, 26 and 26.4°C given by 10 laboratories were not taken into account for this calculation.

## 2. EXPLOITATION OF ANALYSIS REPORT

### 2.1.PREPARATION OF THE INITIAL SUSPENSION

For **338** answers (100%) :

209 laboratories (61.8%) prepare the initial suspension with adding diluent to powder.

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

1 laboratory (0.3%) used another technique to prepare the initial suspension.

### 2.2.DILUENT USED FOR THE INITIAL SUSPENSION

For **335** answers (99.1%) :

281 laboratories (83.1%) use Buffered Peptone Water for the initial suspension.

40 laboratories (11.8%) use Peptone salt for the initial suspension.

14 laboratories (4.1%) used another diluent for the initial suspension. Among these 14 laboratories, 9 specified utilization of Salmonella enrichment broth.

### 2.3.HOMOGENEIZATION TECHNIQUE

For **336** answers (99.4%) :

313 laboratories (92.6%) homogenize their sampling with a Stomacher<sup>ND</sup>.

23 laboratories (6.8%) used another technique (manual, magnétique or other).

### 2.4.RESUSCITATION'S CONDITIONS

#### 2.4.1. DURATION

324 laboratories (95.9%) specified it.

The average duration is **26.7 min** with a standard deviation of 15.5 min. The data 90, 120 and 1440 min given by 3 laboratories were not taken into account for this calculation.

#### 2.4.2. TEMPERATURE

324 laboratories (95.9%) specified it.

The average temperature is **21.3°C** with a standard deviation of 2.7°C.

## 2.5.MICROORGANISM AT 30°C

**321** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 4833-1	197
	AFNOR 3M-01/1-09/89	50
	NM ISO 4833-1	23
	ISO/NF EN ISO 4833-2	16
	AFNOR BIO-12/35-05/13	12
	XP V08-034	6
	Other + V08-100 (spiral)	17 20
<b>Culture medium</b>	Plate Count Agar	233
	Petriefilms	50
	Plate Count Agar + Milk	23
	Tempo AC	12
	Other	1
<b>Preparation</b>	Home made	108
	Ready to use not pre-poured	140
	Ready to use, plate, film, card	71
<b>Plating method</b>	Surface	70
	Pour	230
	Culture medium for card	13
<b>1<sup>st</sup> dilution retained</b>	- 1	18
	- 2	17
	- 3	262
	- 4	12
	1/400	6
	1/4000	4
<b>Incubation temperature</b>	30°C	315
	33-35°C	2
	37°C	1
	25°C	1
<b>Incubation duration</b>	68-75 h	263
	44-48 h	48
	24-26 h	5
	40 h	2
	120 h	1

## 2.6. ENTEROBACTERIACEA

**279** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-054	107
	→ <i>NM 08.0.109</i> <sup>(1)</sup>	20
	ISO/NF EN ISO 21528-2	73
	AFNOR 3M-01/6-09/97	46
	AFNOR BIO-12/21-12/06	11
	AFNOR AES-10/07-01/08	8
	AFNOR BRD-07/24-11/13	8
	Other	6
	+ V08-100 (spiral)	4
<b>Culture medium</b>	VRBG	200
	Petrifilms	49
	Tempo EB	10
	Rebecca	10
	Rapid'Enterobacteriaceae	8
	Other	2
<b>Preparation</b>	Home made	83
	Ready to use not pre-poured	137
	Ready to use, plate, film, card	59
<b>1<sup>st</sup> dilution retained</b>	- 1	212
	- 2	59
	1/40	3
	1/400	5
<b>Incubation temperature</b>	37°C	178
	30°C	89
	35°C	11
<b>Incubation duration</b>	20-26 h	272
	48 h	6
<b>Confirmatory test</b>	Yes	66
	No	208

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

## 2.7.TOTAL COLIFORMS

**237** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-050	109
	→ <i>NM 08.0.142</i> <sup>(2)</sup>	9
	ISO/NF ISO 4832	59
	AFNOR 3M	24
	NM ISO 4832	20
	AFNOR BIO-12/17-12/05	6
	AFNOR BRD-07/08-12/04	4
	Other	6
	+ V08-100 (spiral)	2
<b>Culture medium</b>	VRBL	199
	Petrifilms	25
	Tempo TC	6
	Rapid Ecoli	5
	Other	2
<b>Preparation</b>	Home made	84
	Ready to use not pre-poured	124
	Ready to use, plate, film, card	29
<b>1<sup>st</sup> dilution retained</b>	-1	201
	-2	30
	1/40	2
	1/400	2
<b>Incubation temperature</b>	30°C	219
	37°C	17
<b>Incubation duration</b>	18.1-27 h	233
	48 h	3

AFNOR 3M method including :

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

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

<sup>(2)</sup> *Similar method to NF V 08-050 according to ONSSA.*

## 2.8.THERMOTOLERANT COLIFORMS

**219** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-060	146
	→ NM 08.0.124 <sup>(3)</sup>	27
	AFNOR 3M	28
	ISO/NF ISO 4832	14
	Other	4
	+ V08-100 (spiral)	3
<b>Culture medium</b>	VRBL	188
	Petrifilms	29
	Other	2
<b>Preparation</b>	Home made	79
	Ready to use not pre-poured	114
	Ready to use, plate, film, card	26
<b>1<sup>st</sup> dilution retained</b>	-1	195
	-2	23
<b>Incubation temperature</b>	42-45°C	216
	37°C	2
<b>Incubation duration</b>	21-24 h	214
	48 h	3
	34 h	1

AFNOR 3M method including :

6 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 CC method.

1 laboratory specified utilization of AFNOR 3M Petrifilm high sensitivity method.

1 laboratory specified utilization of AFNOR 3M Petrifilm EC method.

<sup>(3)</sup> *Similar method to NF V08-060 according to ONSSA.*



## 2.9.ESCHERICHIA COLI

**295** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF ISO 16649-2	170
	AFNOR 3M	41
	NM ISO 16649-2	22
	AFNOR BRD-07/01-07/93	18
	AFNOR BIO-12/13-02/05	11
	AFNOR AES-10/06-01/08	9
	NM 08.0.108	6
	AFNOR BIO-12/05-01/99	5
	NF EN ISO 16649-3	1
	Other + V08-100 (spiral)	4
<b>Culture medium</b>	TBX	200
	Petrifilms	42
	Rapid E. coli	21
	Rebecca	12
	Tempo EC	11
	Coli ID	7
	Other	1
<b>Preparation</b>	Home made	84
	Ready to use not pre-poured	159
	Ready to use, plate, film, card	50
<b>Plating method</b>	Surface	43
	Pour	232
	Culture medium for card	12
<b>1<sup>st</sup> dilution retained</b>	-1	275
	-2	9
	1/40	3
	1/400	6
<b>Incubation temperature</b>	41-46°C	257
	37°C	34
	30°C	1
<b>Incubation duration</b>	18-26 h	288
	48 h	4

AFNOR 3M method including :

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

1 laboratory specified utilization of AFNOR 3M-01/04-09/92 method.

1 laboratory specified utilization of AFNOR 3M Petrifilm EC method.

## 2.10. ANAEROBIC SULFITE-REDUCING BACTERIA

**241** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-061	158
	→ <i>NM 08.0.125</i> <sup>(4)</sup>	15
	ISO/NF ISO 15213	41
	NM ISO 15213	13
	Other	14
<b>Culture medium</b>	TSC	220
	TSN	10
	Iron Sulfite agar	7
	Other	3
<b>Preparation</b>	Home made	91
	Ready to use not pre-poured	123
	Ready to use, plate, film, card	27
<b>Seeding way</b>	Plates	150
	Tubes	90
<b>1<sup>st</sup> dilution retained</b>	-1	159
	-2	75
	-3	3
<b>Incubation temperature</b>	44-50°C	173
	37±1°C	65
	30°C	3
<b>Incubation duration</b>	16-24 h	202
	43-48 h	31
	72 h	8

<sup>(4)</sup> *Similar method to NF V08-061 according to ONSSA.*

## 2.11. CLOSTRIDIUM PERFRINGENS

192 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 7937	158
	NM ISO 7937	24
	NM 08.0.111	2
	Other	8
<b>Culture medium</b>	TSC	190
	Other	1
<b>Preparation</b>	Home made	65
	Ready to use not pre-poured	122
	Ready to use, plate, film, card	4
<b>1<sup>st</sup> dilution retained</b>	-1	148
	-2	41
	-3	2
<b>Incubation temperature</b>	37±1°C	181
	44-46°C	9
	30°C	1
<b>Incubation duration</b>	17-27 h	184
	48 h	7
<b>Confirmation test</b>	None	30
	Lactose-sulfite	132
	Strip	9
	Other	6

## 2.12. COAGULASE POSITIVE STAPHYLOCOCCI

298 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 6888-2	139
	ISO/NF EN ISO 6888-1	67
	AFNOR BKR-23/10-12/15	21
	NM ISO 6888-1	20
	AFNOR 3M-01/9-04/03	17
	AFNOR BIO-12/28-04/10	11
	NM ISO 6888-2	5
	NM 08.0.112	3
	NordVal No :049	2
	Other	16
	+ V08-100 (spiral)	6
<b>Culture medium</b>	RPF	135
	BP+egg yolk tellurite	86
	Easy Staph	27
	Petrifilm	18
	BP+egg yolk tellurite+ sulfamethazine	13
	Tempo STA	11
	Rapid Staph	2
	Other	6
<b>Preparation</b>	Home made	65
	Ready to use not pre-poured	126
	Ready to use, plate, film, cards	104
<b>Plating method</b>	Surface	152
	Pour	132
	Culture medium for card	11
<b>1<sup>st</sup> dilution retained</b>	-1	119
	-2	164
	-3	3
	1/40	3
	1/400	5
<b>Incubation temperature</b>	37±1°C	294
	30°C	2
<b>Incubation duration</b>	40-48 h	194
	18-26 h	102
<b>Confirmation test</b>	None	185
	Staphylo-coagulase	78
	Clumping factor	16
	DNase	10
	Other	4

## 2.13. LISTERIA MONOCYTOGENES – ENUMERATION

**244** 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
<b>Method</b>	ISO/NF EN ISO 11290-2	63
	AFNOR AES-10/05-09/06	59
	AFNOR BKR-23/05-12/07	48
	AFNOR BRD-07/05-09/01	29
	NM ISO 11290-2	27
	AFNOR BRD-07/17-01/09	10
	Other	8
<b>Resuscitation medium</b>	Buffered Peptone Water	198
	Fraser base	33
	Other	11
<b>Enumeration medium</b>	ALOA Count	114
	Compass Listeria	69
	Rapid Lmono	33
	AL Agar	16
	Palcam	6
	OCLA	4
	Other	2
<b>Preparation</b>	Home made	36
	Ready to use not pre-poured	49
	Ready to use, plate, film, card	158
<b>Plating method</b>	Surface	200
	Pour	40
	Culture medium for card	0

Parameters	Mode	Nb laboratories
<b>1<sup>st</sup> dilution retained</b>	-1	153
	-2	87
<b>Incubation temperature</b>	37±1°C	239
	30°C	3
	22°C	1
<b>Incubation duration</b>	42-48 h	193
	24±1 h	49
	1 h	1
<b>Confirmation test</b>	None	49
	Biochemical	134
	Biochemical + CAMP	39
	Other	11
<b>Nb of colonies tested per plate</b>	1	63
	2-3	11
	5	106

## 2.14. SALMONELLA – DETECTION

**305** laboratories performed the detection.

Methods used by laboratories are clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 6579-1	78
	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	72
	NM ISO 6579-1	31
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	29
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	29
	AFNOR BIO 12/41-03/17 (SALMA One day)	27
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	20
	AFNOR UNI 03/07-11/13 (PCR)	2
	AFNOR BRD 07/06-07/04 (PCR)	1
	Other	16

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 BIO 12/16-09/05 <b>VIDAS Easy Salmonella</b>	BPW / 37°C - 16/20h	SX2 / 41,5°C - 22/26h	Chrom ID / 37°C - 24h
AFNOR BIO 12/32-10/11 <b>VIDAS SPT</b>		BPW + Salmonella supplement / 41,5°C - 18/24h	Chrom ID / 37°C - 24h
AFNOR BKR 23/07-10/11 <b>IRIS Salmonella</b>		IRIS Salmonella Enrichment / 41,5°C - 18±2h	IRIS / 37°C - 24±3h
AFNOR BRD 07/11-12/05 <b>Rapid Salmonella</b>		BPW + Salmonella capsule / 41,5°C - 18±2h	Rapid Salmonella / 37°C - 24±2h
AFNOR BIO 12/41-03/17 <b>SALMA One day</b>		BPW + Salmonella supplement / 41,5°C - 16/24h	SALMA / 37°C - 24±3h
AFNOR UNI 03/07-11/13 <b>PCR</b>		BPW + supplement / 34-38°C - 20/24h	Lysis + PCR
AFNOR BRD 07/06-07/04 <b>PCR</b>		BPW / 37°C - 18/21h	Lysis + PCR

The detail of the methodology followed by 109 laboratories using ISO/NF EN ISO 6579-1 and NM ISO 6579-1 method, and the 16 laboratories using another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 6579-1	78
	NM ISO 6579-1	31
	Other	16
<b>Pre-enrichment medium</b>	None pre-enrichment	0
	Buffered Peptone Water	118
	Other	5
<b>Pre-enrichment temperature</b>	37±1°C	113
	41-42.5°C	9
	20-22°C	2
<b>Pre-enrichment duration</b>	16-20 h	88
	22-24 h	34
	30 h	1
	72 h	1
<b>Enrichment medium</b>	None enrichment	5
	RVS	107
	MKTTn	105
	Selenite-cystine broth	21
	Other	7
<b>Isolation medium</b>	XLD	107
	Hektoen	33
	Bismuth Sulfate	23
	ASAP	18
	GVB	11
	SS	10
	IRIS Salmonella agar	9
	Rapid Salmonella	9
	Brilliance Salmonella	8
	Compass Salmonella	5
	Rambach	3
	Other	11
<b>Confirmation test</b>	Biochemical	45
	Biochemical + serological agglutination	70
	Other	7



## 2.15. LISTERIA MONOCYTOGENES – DETECTION

271 laboratories performed the detection.

Parameter	Mode	Nb laboratories
<b>Method</b>	AFNOR AES 10/03-09/00 (ALOA one day)	65
	ISO/NF EN ISO 11290-1	54
	AFNOR BKR 23/02-11/02 (Compass L. mono)	49
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	26
	NM ISO 11290-1	23
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	10
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	8
	AFNOR BIO 12/02-06/94 (VIDAS Listeria)	7
	AFNOR BRD 07/16-01/09 (Agar Listeria)	6
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	4
	AFNOR UNI 03/08-11/13 (PCR)	4
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	3
	AFNOR UNI 03/04-04/05 (Listeria Precis)	2
	Other	10

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 BRD 07/04-09/98 <b>Rapid' L. mono</b>	Fraser 1/2	30°C - 24±2h			Rapid L'mono 37°C – 24h
AFNOR BIO 12/02-06/94 <b>VIDAS Listeria</b>	Fraser 1/2	37°C - 26/30h	Fraser	30°C - 24/26h	Palcam et Oxford 37°C – 24h
AFNOR BIO 12/27-02/10 <b>VIDAS LMX</b>	LMX	37°C - 26/30h			ChromID 37°C – 24h
AFNOR BIO 12/11-03/04 <b>VIDAS LMO2 (37°C)</b>	Fraser 1/2	30°C - 24/26h	Fraser	37°C - 24/26h	Chromogenic medium / Palcam / Oxford
AFNOR AES 10/03-09/00 <b>ALOA one day</b>	Fraser 1/2	30°C - 24±2h			ALOA One Day 37°C – 24/48h
AFNOR BKR 23/02-11/02 <b>Compass L. mono</b>	Fraser 1/2	30°C - 24±2h			Compass Listeria Agar 37°C – 24h
AFNOR BRD 07/16-01/09 <b>Agar Listeria</b>	Fraser 1/2	30°C - 24±2h			Agar Listeria 37°C – 24h
AFNOR UNI 03/04-04/05 <b>Listeria Precis</b>	One Broth Listeria	30°C - 24±2h			Brilliance Listeria 37°C – 24h
AFNOR BIO 12/40-11/16 <b>GENE UP LMO</b>	LPT	35-37°C - 24±2h			ALOA 35-37°C – 24/48h
AFNOR BIO 12/18-03/06 <b>VIDAS LDUO</b>	LX	30°C - 24±2h	LX	30°C - 24/26h	Chromogenic medium / Palcam / Oxford
AFNOR UNI 03/08-11/13 <b>PCR</b>	LEB	37°C - 24/28h			Lysis + PCR

The detail of the methodology followed by 77 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 methods, and the 10 laboratories using another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 11290-1	54
	NM ISO 11290-1	23
	Other	10
<b>Primary enrichment medium</b>	None primary enrichment	1
	Half-Fraser	76
	One broth Listeria	3
	Other	7
<b>Primary enrichment temperature</b>	30°C	82
	37°C	4
<b>Primary enrichment duration</b>	22-27 h	85
	48 h	1
<b>Secondary enrichment medium</b>	None secondary enrichment	9
	Fraser	73
	Other	1
<b>Secondary enrichment temperature</b>	37°C	71
	30°C	2
<b>Secondary enrichment duration</b>	22-25 h	59
	48 h	14
<b>Isolation medium</b>	Palcam	56
	Ottaviani et Agosti	44
	Compass Listeria	30
	Oxford	11
	Rapid L'mono	4
	Brilliance Listeria	3
	Other	2
<b>Isolation temperature</b>	37°C	80
	30°C	3
<b>Isolation duration</b>	44-48 h	51
	24±1 h	32
<b>Confirmation test</b>	None	6
	Biochemical	54
	Biochemical + CAMP	20
	Other	4
<b>Nb of colonies per plate</b>	1	29
	2-3	7
	5	38
	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 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 characterized by its own assigned value.

A z score is then calculated with the following formula :  $z = \frac{m - m_{pt}}{\sigma_{pt}}$ , where  $\sigma_{pt}$  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 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.

<b>Microorganisms at 30°C</b>	
Assigned value of the contamination (log cfu/g)	4.870
Assigned value uncertainty (log cfu/g)	0.0062
Standard deviation for proficiency assessment (log cfu/g)	0.0845
Standard deviation for precision (log cfu/g)	0.0573
Interlaboratory's standard deviation (log cfu/g)	0.0805
Reproducibility standard deviation (log cfu/g)	0.0989

### 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 three groups :

<b>Enterobacteriaceae</b>	Group 1	Group 2	Group 3
Assigned value of the contamination (log cfu/g)	2.658	2.920	3.180
Assigned value uncertainty (log cfu/g)	0.0258	0.0663	0.0179
Standard deviation for proficiency assessment (log cfu/g)	0.2808	0.2652	0.0972
Standard deviation for precision (log cfu/g)	0.1168		
Interlaboratory's standard deviation (log cfu/g)	0.2759	0.2600	0.0820
Reproducibility standard deviation (log cfu/g)	0.2997	0.2850	0.1427

### 3.1.3. TOTAL COLIFORMS

A significant "effect" of the culture medium, manufacturer, and the preparation mode 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 :

<b>Total coliforms</b>	Group 1	Group 2	Group 3
Assigned value of the contamination (log cfu/g)	2.416	2.658	3.160
Assigned value uncertainty (log cfu/g)	0.0403	0.0305	0.0368
Standard deviation for proficiency assessment (log cfu/g)	0.2659	0.2688	0.1350
Standard deviation for precision (log cfu/g)	0.1154		
Interlaboratory's standard deviation (log cfu/g)	0.2609	0.2638	0.1248
Reproducibility standard deviation (log cfu/g)	0.2858	0.2885	0.1709

### 3.1.4. THERMOTOLERANT COLIFORMS

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 three groups :

<b>Thermotolerant coliforms</b>	Group 1	Group 2	Group 3
Assigned value of the contamination (log cfu/g)	2.469	2.626	2.912
Assigned value uncertainty (log cfu/g)	0.0252	0.0400	0.0924
Standard deviation for proficiency assessment (log cfu/g)	0.2101	0.2617	0.3388
Standard deviation for precision (log cfu/g)	0.1238		
Interlaboratory’s standard deviation (log cfu/g)	0.2027	0.2558	0.3343
Reproducibility standard deviation (log cfu/g)	0.2339	0.2812	0.3541

### 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 :

<b><i>Escherichia coli</i></b>	
Assigned value of the contamination (log cfu/g)	2.188
Assigned value uncertainty (log cfu/g)	0.0173
Standard deviation for proficiency assessment (log cfu/g)	0.2247
Standard deviation for precision (log cfu/g)	0.1822
Interlaboratory’s standard deviation (log cfu/g)	0.2094
Reproducibility standard deviation (log cfu/g)	0.2775

### 3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°1, 2 and 5 were artificially contaminated.

A significant “effect” of the manufacturer and the retained dilution has been highlighted. This effect results in a contamination’s difference lower than 0.3 log cfu/g, then results have been gathered in one group :

<b>Anaerobic sulfite-reducing bacteria</b>	
Assigned value of the contamination (log cfu/g)	2.764
Assigned value uncertainty (log cfu/g)	0.0200
Standard deviation for proficiency assessment (log cfu/g)	0.2379
Standard deviation for precision (log cfu/g)	0.0914
Interlaboratory’s standard deviation (log cfu/g)	0.2319
Reproducibility standard deviation (log cfu/g)	0.2493

Comment :

- 5 laboratories detected ASR in unit n°3 non-artificially contaminated by *C. perfringens* with a contamination level from 20 to 1600 cfu/g.

- 5 laboratories detected ASR in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 10 to 2000 cfu/g.

### 3.1.7. CLOSTRIDIUM PERFRINGENS

Only units n°1, 2 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 :

<b><i>Clostridium perfringens</i></b>	
Assigned value of the contamination (log cfu/g)	2.762
Assigned value uncertainty (log cfu/g)	0.0214
Standard deviation for proficiency assessment (log cfu/g)	0.2277
Standard deviation for precision (log cfu/g)	0.0929
Interlaboratory’s standard deviation (log cfu/g)	0.2213
Reproducibility standard deviation (log cfu/g)	0.2400

Comment :

- 4 laboratories detected *C. perfringens* in unit n°3 non-artificially contaminated by *C. perfringens* with a contamination level from 400 to 1600 cfu/g.
- 1 laboratory detected *C. perfringens* in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level of 1000 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 :

<b>Coagulase positive Staphylococci</b>	
Assigned value of the contamination (log cfu/g)	3.612
Assigned value uncertainty (log cfu/g)	0.0112
Standard deviation for proficiency assessment (log cfu/g)	0.1490
Standard deviation for precision (log cfu/g)	0.0802
Interlaboratory’s standard deviation (log cfu/g)	0.1446
Reproducibility standard deviation (log cfu/g)	0.1654

### 3.1.9. LISTERIA MONOCYTOGENES

Only units n°1, 2 and 5 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

<b><i>Listeria monocytogenes</i></b>	
Assigned value of the contamination (log cfu/g)	3.408
Assigned value uncertainty (log cfu/g)	0.0086
Standard deviation for proficiency assessment (log cfu/g)	0.1040
Standard deviation for precision (log cfu/g)	0.0732
Interlaboratory’s standard deviation (log cfu/g)	0.0950
Reproducibility standard deviation (log cfu/g)	0.1200

### 3.2.PERFORMANCES IN DETECTION

The performance is assessed by the capacity to detect only samples contaminated by *Salmonella* and *Listeria monocytogenes* (no false positive or false negative results).

#### 3.2.1. DETECTION – SALMONELLA

Only units n°1 and 5 were artificially contaminated.

294 laboratories obtained correct results.

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

6 laboratories obtained false negative results (respectively 3 and 4 false-negative for units n° 1 and 5).

#### 3.2.2. DETECTION – LISTERIA MONOCYTOGENES

Only units n°1, 2 and 5 were artificially contaminated.

266 laboratories obtained correct results.

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

2 laboratories obtained false negative results (respectively 1, 1 and 2 false-negative for units n°1, 2 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 53<sup>th</sup> 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.