

## PROFICIENCY TEST « RAEMA »



### POWDER SCHEME N° 81 (30<sup>th</sup> SEPTEMBER 2025) GENERAL REPORT

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## 1- GENERAL DATA

### 1.1. PARTICIPATING LABORATORIES

**322 laboratories** participated to the 81<sup>th</sup> scheme. The sending was made on Tuesday 30<sup>th</sup> September 2025.

We received **316** answers (98.1%).

### 1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J0+9	J0+10	J0+11	J0+13
Nb of laboratories	6	203	58	25	3	9	3	4	1	2	1	1

### 1.3. INFORMATIONS ABOUT SAMPLE

#### 1.3.1. NATURE

The sample included :

- one strain of *Enterococcus sp.* at a concentration level of  $1.10^5$  cfu/g in 5 units ;
- one strain of *Citrobacter sp.* at a concentration level of  $1,5.10^3$  cfu/g in 5 units ;
- one strain of *Serratia marcescens* at a concentration level of  $7.10^2$  cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of  $5.10^2$  cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of  $2,5.10^2$  cfu/g in 2 units ;
- one strain of *Staphylococcus aureus* at a concentration level of  $2.10^3$  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  $1.10^3$  cfu/g in 3 units.

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

#### 1.3.2. SIZE

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

#### 1.3.3. HOMOGENEITY AND STABILITY TEST OF THE CONTAMINATION

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

The contamination's stability was also checked by enumeration / detection of all flora on 6, 13 and 20 October 2025. These checks were realized by an external provider 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

316 laboratories (100%) specified it.

Beginning of analysis	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+13	J0+14	J0+15
Nb of laboratories	31	35	17	3	2	136	56	14	4	1	8	8	1

### 1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

316 laboratories (100%) specified it. The average temperature is **4.2°C** with a standard deviation of 1.0°C. The given data 20, 23.9 and 30°C given by 5 laboratories were not taken into account for this calculation.

## 2. EXPLOITATION OF ANALYSIS REPORT

### 2.1. SIZE OF THE SAMPLES

314 laboratories specified it (99.4%).

The average size is **17.9 g** with a standard deviation of 8.1 g. The minimum size indicated is 1 g and the maximum one is 60 g.

### 2.2. PREPARATION OF THE INITIAL SUSPENSION

For 315 answers (99.7%) :

197 laboratories (62.3%) prepare the initial suspension with adding diluent to powder.

118 laboratories (37.4%) prepare the initial suspension with adding powder to diluent.

### 2.3. DILUENT USED FOR THE INITIAL SUSPENSION

For 315 answers (99.7%) :

276 laboratories (87.3%) use Buffered Peptone Water (or equivalent) for the initial suspension.

34 laboratories (10.8%) use Peptone salt for the initial suspension.

5 laboratories (1.6%) used another diluent for the initial suspension.

### 2.4. HOMOGENEIZATION TECHNIQUE

For 313 answers (99.1%) :

284 laboratories (89.9%) homogenize their sampling with a Stomacher<sup>ND</sup>.

22 laboratories (7.0%) used a manual homogenization.

5 laboratories (1.6%) used a Vortex mixer.

2 laboratories (0.6%) used another technique.

### 2.5. RESUSCITATION'S CONDITIONS

#### 2.5.1. DURATION

300 laboratories (94.9%) specified it.

The average duration is **25.9 min** with a standard deviation of 15.1 min. The data 120 and 180 min given by two laboratories was not taken into account for this calculation.

#### 2.5.2. TEMPERATURE

300 laboratories (94.9%) specified it.

The average temperature is **21.9°C** with a standard deviation of 3.9°C.

## 2.6. MICROORGANISM AT 30°C

**302** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 4833-1 (+A1)	185
	AFNOR 3M-01/1-09/89	42
	NM ISO 4833-1	30
	ISO/NF EN ISO 4833-2 (+A1)	14
	AFNOR BIO-12/35-05/13	12
	XP V08-034	7
	Internal method	5
	Other	7
	+ Spiral metho	19
<b>Culture medium</b>	Plate Count Agar	228
	Neogen® Petrifilms®	43
	Plate Count Agar + Milk	18
	Tempo AC	12
	Other	1
<b>Preparation</b>	Home made	107
	Ready to use not pre-poured	125
	Ready to use, plate, film, card	69
<b>Plating method</b>	Surface	56
	Pour	224
	Transfer Tempo filler®	12
<b>1<sup>st</sup> dilution retained</b>	- 1	14
	- 2	16
	- 3	247
	- 4	16
	- 5	1
	1/400	4
	1/4000	2
<b>Incubation temperature</b>	30°C	298
	32-33°C	2
	37°C	2
<b>Incubation duration</b>	69-72 h	251
	40-48 h	47
	120 h	2
	26 h	1

## 2.7. ENTEROBACTERIACEAE

**276** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-054	100
	→ <i>NM 08.0.109</i> <sup>(1)</sup>	15
	ISO/NF EN ISO 21528-2	72
	AFNOR 3M-01/6-09/97	42
	NM ISO 21528-2	20
	AFNOR BIO-12/21-12/06	10
	AFNOR BRD-07/24-11/13	8
	AFNOR AES-10/07-01/08	6
	Internal method	2
	Other	1
<b>Culture medium</b>	VRBG	202
	Neogen® Petrifilms®	46
	Tempo EB	10
	Rapid'Enterobacteriaceae	9
	Rebecca	8
<b>Preparation</b>	Home made	84
	Ready to use not pre-poured	133
	Ready to use, plate, film, card	57
<b>1<sup>st</sup> dilution retained</b>	- 1	127
	- 2	142
	1/40	2
	1/400	4
<b>Incubation temperature</b>	37-37.5°C	178
	30-32°C	87
	35°C	10
<b>Incubation duration</b>	20-26 h	271
	48 h	3
<b>Confirmatory test</b>	Yes	67
	No	203

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

## 2.8. TOTAL COLIFORMS

**214** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-050	97
	→ <i>NM 08.0.142</i> <sup>(2)</sup>	9
	ISO/NF ISO 4832	51
	NM ISO 4832	26
	AFNOR 3M	13
	AFNOR BIO-12/17-12/05	7
	AFNOR BRD-07/08-12/04	6
	Internal method	1
	Other	4
<b>Culture medium</b>	VRBL	183
	Neogen® Petrifilms®	14
	Rapid Ecoli 2	7
	Tempo TC	7
	Other	3
<b>Preparation</b>	Home made	83
	Ready to use not pre-poured	107
	Ready to use, plate, film, card	22
<b>1<sup>st</sup> dilution retained</b>	-1	139
	-2	70
	1/40	1
	1/400	3
<b>Incubation temperature</b>	30-32°C	195
	35-37°C	18
<b>Incubation duration</b>	20-24 h	205
	44-48 h	7
	30 h	1

AFNOR 3M method including :

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

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



## 2.9. THERMOTOLERANT COLIFORMS

**188** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-060	119
	→ <i>NM 08.0.124</i> <sup>(3)</sup>	34
	AFNOR 3M	19
	ISO/NF ISO 4832	14
	Other	2
<b>Culture medium</b>	VRBL	166
	Neogen® Petrifilms®	19
	Other	3
<b>Preparation</b>	Home made	79
	Ready to use not pre-poured	90
	Ready to use, plate, film, card	18
<b>1<sup>st</sup> dilution retained</b>	-1	168
	-2	18
	-3	1
<b>Incubation temperature</b>	42-44.5°C	186
	37°C	2
<b>Incubation duration</b>	22-24 h	183
	48 h	4
	30 h	1

AFNOR 3M method including :

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

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

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

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

## 2.10. ESCHERICHIA COLI

**285** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF ISO 16649-2	173
	AFNOR 3M	37
	NM ISO 16649-2	29
	AFNOR BRD-07/01-07/93	13
	AFNOR BIO-12/13-02/05	10
	NM 08.0.108	5
	AFNOR AES-10/06-01/08	5
	AFNOR BRD-07/07-12/04	5
	AFNOR BIO-12/05-01/99	2
	Internal method	2
	ISO/NF EN ISO 16649-3	1
	Other	3
<b>Culture medium</b>	TBX	203
	Neogen® Petrifilms®	38
	Rapid E. coli 2	19
	Tempo EC	10
	Rebecca	8
	Coli ID	4
	Other	3
<b>Preparation</b>	Home made	89
	Ready to use not pre-poured	143
	Ready to use, plate, film, card	49
<b>Plating method</b>	Surface	41
	Pour	226
	Transfer Tempo filler®	10
<b>1<sup>st</sup> dilution retained</b>	-1	257
	-2	22
	1/40	1
	1/400	3
<b>Incubation temperature</b>	41-46°C	256
	37°C	24
	30-32°C	2
<b>Incubation duration</b>	18-26 h	279
	48 h	4

## 2.11. ANAEROBIC SULFITE-REDUCING BACTERIA

**219** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF V08-061	135
	→ <i>NM 08.0.125</i> <sup>(4)</sup>	21
	ISO/NF ISO 15213-1	41
	NM ISO 15213-1	11
	Internal method	4
	Other	5
<b>Culture medium</b>	TSC	186
	Iron Sulfite agar	26
	TSN	5
	Other	1
<b>Preparation</b>	Home made	80
	Ready to use not pre-poured	118
	Ready to use, plate, film, card	20
<b>Seeding way</b>	Plates	155
	Tubes	63
<b>1<sup>st</sup> dilution retained</b>	-1	177
	-2	39
	-3	1
<b>Incubation temperature</b>	44-46°C	158
	37°C	60
<b>Incubation duration</b>	14-24 h	173
	46-48 h	40
	72 h	5

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

## 2.12. CLOSTRIDIUM PERFRINGENS

**185** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF ISO 15213-2	93
	ISO/NF EN ISO 7937 ( <i>repealed</i> )	54
	NM ISO 15213-2	22
	NM ISO 7937 ( <i>repealed</i> )	5
	Internal method	3
	Other	6
<b>Culture medium</b>	TSC	182
	Other	3
<b>Preparation</b>	Home made	62
	Ready to use not pre-poured	118
	Ready to use, plate, film, card	5
<b>1<sup>st</sup> dilution retained</b>	-1	163
	-2	22
<b>Incubation temperature</b>	36-37.5°C	178
	44-46°C	6
<b>Incubation duration</b>	18-24 h	175
	48 h	9
<b>Confirmation test</b>	None	27
	SIM agar	86
	Lactose-sulfite	45
	Acid phosphatase	8
	MALDI-TOF mass spectrometry	5
	Strip	1
	Other	3

\*Comment: ISO 7937 methods have been repealed and replaced by ISO 15213-2 methods since November 2023.

## 2.13. COAGULASE POSITIVE STAPHYLOCOCCI

**281** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 6888-2 (+A1)	126
	ISO/NF EN ISO 6888-1 (+A1)	62
	AFNOR BKR-23/10-12/15	26
	NM ISO 6888-1	23
	AFNOR BIO-12/28-04/10	12
	AFNOR 3M-01/09-04/03	10
	NM ISO 6888-2	7
	Internal method	3
	NM 08.0.112	3
	ISO/NF EN ISO 6888-3	2
	NordVal No :049	2
	Other	3
<b>Culture medium</b>	RPF	125
	BP+egg yolk tellurite	86
	Easy Staph	30
	Tempo STA	12
	Neogen® Petrifilm®	11
	BP+egg yolk tellurite+ sulfamethazine	9
	Rapid Staph	3
	Other	3
<b>Preparation</b>	Home made	75
	Ready to use not pre-poured	113
	Ready to use, plate, film, cards	86
<b>Plating method</b>	Surface	135
	Pour	127
	Transfer Tempo filler®	12
<b>1<sup>st</sup> dilution retained</b>	-1	136
	-2	136
	-3	2
	1/40	4
	1/400	2
<b>Incubation temperature</b>	35-37.5°C	278
	30°C	1
<b>Incubation duration</b>	42-48 h	185
	18-27 h	93
	32 h	1
<b>Confirmation test</b>	None	173
	Staphylo-coagulase	68
	Clumping factor	21
	DNase	5
	MALDI-TOF mass spectrometry	4
	Other	2

## 2.14. LISTERIA MONOCYTOGENES – ENUMERATION

**221** laboratories performed the enumeration.

### RESUSCITATION

56 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	58
	AFNOR BKR-23/05-12/07	55
	AFNOR AES-10/05-09/06	48
	NM ISO 11290-2	27
	AFNOR BRD-07/05-09/01	22
	AFNOR BRD-07/17-01/09	8
	Internal method	1
	Other	2
<b>Resuscitation medium</b>	Buffered Peptone Water or equivalent	173
	Half-fraser	35
	Fraser base	3
	Other	5
<b>Enumeration medium</b>	ALOA Count	98
	Compass Listeria	84
	Rapid Lmono	22
	AL Agar	10
	Palcam	4
	OCLA	2
	Brilliance Listeria	1
<b>Preparation</b>	Home made	40
	Ready to use not pre-poured	47
	Ready to use, plate, film, card	134
<b>Plating method</b>	Surface	182
	Pour	38

Parameters	Mode	Nb laboratories
<b>1<sup>st</sup> dilution retained</b>	-1	187
	-2	31
<b>Incubation temperature</b>	37-37.5°C	216
	30°C	5
<b>Incubation duration</b>	42-48 h	189
	22-24 h	32
<b>Confirmation test</b>	None	44
	Biochemical	132
	Biochemical + CAMP	33
	MALDI-TOF mass spectrometry	7
	Other	3
<b>Nb of colonies tested per plate</b>	1	58
	2-3	9
	5	96
	10-12	2
	150	3

## 2.15. SALMONELLA – DETECTION

**287** laboratories performed the detection.

Methods used by laboratories are clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	71
	ISO/NF EN ISO 6579-1 (+A1)	70
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	36
	NM ISO 6579-1	35
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	23
	AFNOR BIO 12/41-03/17 (SALMA One day)	18
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	10
	AFNOR BIO 12/01-04/94 (VIDAS SLM)	7
	AFNOR UNI 03/06-12/07 (Salmonella precis)	3
	AFNOR BIO 12/38-06/16 (GENE UP Salmonella)	3
	AFNOR BRD 07/06-07/04 (PCR)	3
	AFNOR UNI 03/07-11/13 (PCR)	3
	Internal method	1
	AFNOR TRA 02/12-01/09 (Assurance GDS Salmonella Tq)	1
	Other	2

No detail of methodology was asked to laboratories using other methods than ISO/NF EN ISO 6579-1 (+A1) and NM ISO 6579-1 methods and proposed in the questionnaire.

You will find below a short description of these methods :

Method	Pre-enrichment	Enrichment	Isolation
AFNOR BKR 23/07-10/11 <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 supplement / 41,5°C - 18±2h	Rapid Salmonella / 37°C - 24±2h
AFNOR BIO 12/32-10/11 <b>VIDAS SPT</b>		BPW + Salmonella supplement / 41,5°C - 18/24h	Chrom ID / 37°C - 24h
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 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 12/01-04/94 <b>VIDAS SLM</b>	BPW / 35°C – 24±2h	Tetrathionate (42°C - 6/8h) – Selenite cystine (35-37°C – 6/8h) + M-Broth (42°C – 18h)	Vidas Heat & Go
AFNOR UNI 03/06-12/07 <b>Salmonella precis</b>		One broth-Salmonella / 42°C – 16/24h	Brilliance Salmonella / 37°C – 24±2h
AFNOR BIO 12/38-06/16 <b>GENE UP Salmonella</b>		BPW / 42°C – 18/24h	Lysis + PCR
AFNOR BRD 07/06-07/04 <b>PCR</b>		BPW / 37°C – 18/21h	Lysis + PCR
AFNOR UNI 03/07-11/13 <b>PCR</b>		BPW + supplement / 34-38°C – 20/24h	Lysis + PCR
AFNOR TRA 02/12-01/09 <b>Assurance GDS for Salmonella Tq</b>		EPT / 37°C - 18/24h	Amplification + detection
AFNOR TRA 02/08-03/01 <b>TRANSIA PLATE Salmonella GOLD</b>	BPW / 37°C – 16/20h	RVS / 41.5°C – 18/24h	ELISA test
AFNOR QUA 18/03-11/02 <b>BAX SYSTEM PCR</b>		BPW / 37°C – 16/20h	Lysis + PCR





The detail of the methodology followed by 105 laboratories using ISO/NF EN ISO 6579-1 (+A1) and NM ISO 6579-1 methods, and the 3 laboratories using an internal or another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>	ISO/NF EN ISO 6579-1 (+A1)	70
	NM ISO 6579-1	35
	Internal method	1
	Other	2
<b>Pre-enrichment medium</b>	None pre-enrichment	1
	Buffered Peptone Water	105
	Other	2
<b>Pre-enrichment temperature</b>	35-38°C	105
	22°C	2
	41.5°C	1
<b>Pre-enrichment duration</b>	15-20 h	76
	22-24 h	32
<b>Enrichment medium</b>	None enrichment	2
	RVS	102
	MKTTn	99
	Selenite-cystine broth	27
	Other	4
<b>Isolation medium</b>	XLD	101
	Hektoen	33
	Bismuth Sulfate	27
	GVB	13
	IRIS Salmonella agar	11
	ASAP	11
	SS	7
	Rapid Salmonella	5
	Compass Salmonella	3
	Rambach	3
	Brilliance Salmonella	2
	Other	10
<b>Confirmation test</b>	Biochemical	41
	Biochemical + serological agglutination	57
	MALDI-TOF mass spectrometry	7
	Other	1

## 2.16. LISTERIA MONOCYTOGENES – DETECTION

**257** laboratories performed the detection.

Parameter	Mode	Nb laboratories
<b>Method</b>	AFNOR BKR 23/02-11/02 (Compass L. mono)	59
	ISO/NF EN ISO 11290-1	54
	AFNOR AES 10/03-09/00 (ALOA one day)	45
	NM ISO 11290-1	26
	AFNOR BRD 07/04-09/98 (Rapid' L. mono)	21
	AFNOR BRD 07/16-01/09 (Agar Listeria)	11
	AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)	9
	AFNOR BIO 12/27-02/10 (VIDAS LMX)	8
	AFNOR BIO 12/40-11/16 (GENE UP LMO)	6
	AFNOR UNI 03/04-04/05 (Listeria Precis)	5
	Internal method	3
	AFNOR BIO 12/18-03/06 (VIDAS LDUO)	2
	AFNOR UNI 03/08-11/13 (PCR)	2
	AFNOR BRD 07/10-04/05 (IQ Check Listeria)	1
	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 <b>Compass L. mono</b>	Half-Fraser	30°C - 24±2h			Compass Listeria Agar 37°C – 24h
AFNOR AES 10/03-09/00 <b>ALOA one day</b>	Half-Fraser	30°C - 24±2h			ALOA One Day 37°C – 24/48h
AFNOR BRD 07/04-09/98 <b>Rapid' L. mono</b>	Half-Fraser	30°C - 24±2h			Rapid L'mono 37°C – 24h
AFNOR BRD 07/16-01/09 <b>Agar Listeria</b>	Half-Fraser	30°C - 24±2h			Agar Listeria 37°C – 24h
AFNOR BIO 12/11-03/04 <b>VIDAS LMO2 (37°C)</b>	Half-Fraser	30°C - 24/26h	Fraser	37°C - 24/26h	Chromogenic medium / Palcam / Oxford
AFNOR BIO 12/27-02/10 <b>VIDAS LMX</b>	LMX	37°C - 26/30h			ChromID 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 UNI 03/04-04/05 <b>Listeria Precis</b>	One Broth Listeria	30°C - 24±2h			Brilliance Listeria 37°C – 24h
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
AFNOR BRD 07/10-04/05 <b>IQ Check Listeria</b>	Half-Fraser / LSB	30°C – 23/25h			Lysis + PCR
AOAC 070702 <b>Assurance GDS for Listeria monocytogenes</b>	Fraser 1/2	30°C – 22/26h			Amplification + detection

The detail of the methodology followed by 80 laboratories using NF ISO/EN ISO 11290-1 and NM ISO 11290-1 methods, and the 8 laboratories using an internal or 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	26
	Internal method	3
	Other	5
<b>Primary enrichment medium</b>	None primary enrichment	2
	Half-Fraser	82
	Other	3
<b>Primary enrichment temperature</b>	30°C	81
	37°C	4
<b>Primary enrichment duration</b>	18-25 h	85
<b>Secondary enrichment medium</b>	None secondary enrichment	7
	Fraser	81
<b>Secondary enrichment temperature</b>	37±1°C	74
	30°C	5
<b>Secondary enrichment duration</b>	22-24 h	69
	48 h	9
	30 h	1
<b>Isolation medium</b>	Palcam	55
	Ottaviani et Agosti	48
	Compass Listeria	38
	Oxford	13
	Rapid L'mono	5
	Brilliance Listeria	1
<b>Isolation temperature</b>	35-37°C	85
	30°C	2
<b>Isolation duration</b>	48 h	55
	22-24 h	31
	34 h	1
<b>Confirmation test</b>	None	3
	Biochemical	48
	Biochemical + CAMP	28
	MALDI-TOF mass spectrometry	4
	Other	2
<b>Nb of colonies per plate</b>	1	27
	2-3	5
	5	43
	10	1

### 3. ASSESSMENT OF PERFORMANCE (INDIVIDUEL REPORTS)

#### 3.1. PERFORMANCES IN ENUMERATION

Performance is assessed on two criteria : **precision and trueness**.

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

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

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

#### PRECISION

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

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

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

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

As an indicator, we suggest following values by analogy with those indicated for the evaluation of trueness. For  $k=5$ , a score lower than 0.1 or higher than 18 may be considered as an action signal and a score lower than 0.45 or higher than 11.5 may be considered as a warning signal.

For  $k=4$ , a score lower than 0.03 or higher than 15.5 may be considered as an action signal and a score lower than 0.2 or higher than 9.5 may be considered as a warning signal.

For  $k=3$ , a score lower than 0.003 or higher than 13.2 may be considered as an action signal and a score lower than 0.05 or higher than 7.5 may be considered as a warning signal.

For  $k=2$ , a score lower than 0.000002 or higher than 10.3 may be considered as an action signal and a score lower than 0.0008 or higher than 5.2 may be considered as a warning signal.

## TRUENESS

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

The mean of your results in log CFU/g,  $m$  (on contaminated units and included in the statistical analysis), is compared to the contamination's assigned value,  $m_{pt}$ , obtained with algorithm A from the standard NF ISO 13528 applied to all laboratories mean included in the statistical analysis. When groups are formed, each one is 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  $\sigma_{pt}$ , 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  $\sigma_{pt}$  is the standard deviation for proficiency assessment (robust estimation of the standard deviation obtained by participants).

The standard NF ISO 13528 specifies that:

- $|z| \leq 2,0$  is considered as satisfactory (acceptable),
- $2,0 < |z| < 3,0$  is considered as a warning signal,
- $|z| \geq 3,0$  is considered as an action signal (or not acceptable).

The ranges of concentrations values expected to be satisfactory are mentioned in this report for each of the flora proposed for enumeration.

In this report, we also 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)	5.146
Assigned value uncertainty (log cfu/g)	0.0045
Standard deviation for proficiency assessment (log cfu/g)	0.0600
Range of expected satisfactory values (log cfu/g)	[5.026 ; 5.266]
Standard deviation for precision (log cfu/g)	0.0491
Interlaboratory's standard deviation (log cfu/g)	0.0558
Reproducibility standard deviation (log cfu/g)	0.0744

### 3.1.2. ENTEROBACTERIACEAE

A significant "effect" of the manufacturer of the diluent, the method, the culture medium, the manufacturer 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 (64 laboratories)	Group 2 (193 laboratories)
Assigned value of the contamination (log cfu/g)	3.015	3.341
Assigned value uncertainty (log cfu/g)	0.0333	0.0211
Standard deviation for proficiency assessment (log cfu/g)	0.2134	0.2351
Range of expected satisfactory values (log cfu/g)	[2.588 ; 3.442]	[2.871 ; 3.811]
Standard deviation for precision (log cfu/g)	0.0866	
Interlaboratory's standard deviation (log cfu/g)	0.2099	0.2318
Reproducibility standard deviation (log cfu/g)	0.2270	0.2475

### 3.1.3. TOTAL COLIFORMS

A significant "effect" of the manufacturer of the diluent, the method, the culture medium, the manufacturer 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 (23 laboratories)	Group 2 (158 laboratories)	Group 3 (17 laboratories)
Assigned value of the contamination (log cfu/g)	2.811	3.157	3.456
Assigned value uncertainty (log cfu/g)	0.0777	0.0247	0.0623
Standard deviation for proficiency assessment (log cfu/g)	0.2981	0.2482	0.2056
Range of expected satisfactory values (log cfu/g)	[2.215 ; 3.407]	[2.661 ; 3.653]	[3.045 ; 3.867]
Standard deviation for precision (log cfu/g)	0.0783		
Interlaboratory's standard deviation (log cfu/g)	0.2960	0.2458	0.2026
Reproducibility standard deviation (log cfu/g)	0.3084	0.2606	0.2203

**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). All laboratories included in the group 3 obtain a satisfactory z-score (without impact).

### 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)	2.874
Assigned value uncertainty (log cfu/g)	0.0197
Standard deviation for proficiency assessment (log cfu/g)	0.2081
Range of expected satisfactory values (log cfu/g)	[2.458 ; 3.290]
Standard deviation for precision (log cfu/g)	0.0817
Interlaboratory's standard deviation (log cfu/g)	0.2049
Reproducibility standard deviation (log cfu/g)	0.2206

### 3.1.5. ESCHERICHIA COLI

A significant "effect" of the preparation 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)	2.785
Assigned value uncertainty (log cfu/g)	0.0166
Standard deviation for proficiency assessment (log cfu/g)	0.2152
Range of expected satisfactory values (log cfu/g)	[2.355 ; 3.215]
Standard deviation for precision (log cfu/g)	0.0772
Interlaboratory's standard deviation (log cfu/g)	0.2124
Reproducibility standard deviation (log cfu/g)	0.2260

### 3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°1 and 3 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

Anaerobic sulfite-reducing bacteria	
Assigned value of the contamination (log cfu/g)	2.361
Assigned value uncertainty (log cfu/g)	0.0141
Standard deviation for proficiency assessment (log cfu/g)	0.1568
Range of expected satisfactory values (log cfu/g)	[2.047 ; 2.675]
Standard deviation for precision (log cfu/g)	0.0984
Interlaboratory's standard deviation (log cfu/g)	0.1405
Reproducibility standard deviation (log cfu/g)	0.1716

#### Comment :

- 9 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 500 to 3100 cfu/g.



- 10 laboratories detected ASR in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 330 to 5500 cfu/g.
- 9 laboratories detected ASR in unit n°5 non-artificially contaminated by *C. perfringens* with a contamination level from 150 to 8500 cfu/g.

### 3.1.7. CLOSTRIDIUM PERFRINGENS

Only units n°1 and 3 were artificially contaminated.

None significant effect of the analysis technique has been highlighted.

<b><i>Clostridium perfringens</i></b>	
Assigned value of the contamination (log cfu/g)	2.363
Assigned value uncertainty (log cfu/g)	0.0169
Standard deviation for proficiency assessment (log cfu/g)	0.1760
Range of expected satisfactory values (log cfu/g)	[2.011 ; 2.715]
Standard deviation for precision (log cfu/g)	0.1008
Interlaboratory's standard deviation (log cfu/g)	0.1609
Reproducibility standard deviation (log cfu/g)	0.1898

#### **Comment :**

- 5 laboratories detected *C. perfringens* in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 90 to 1200 cfu/g.
- 4 laboratories detected *C. perfringens* in unit n°4 non-artificially contaminated by *C. perfringens* with a contamination level from 170 to 1600 cfu/g.
- 4 laboratories detected *C. perfringens* in unit n°5 non-artificially contaminated by *C. perfringens* with a contamination level from 200 to 1000 cfu/g.

### 3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

None significant effect of the analysis technique has been highlighted.

<b>Coagulase positive Staphylococci</b>	
Assigned value of the contamination (log cfu/g)	3.385
Assigned value uncertainty (log cfu/g)	0.0128
Standard deviation for proficiency assessment (log cfu/g)	0.1657
Range of expected satisfactory values (log cfu/g)	[3.054 ; 3.716]
Standard deviation for precision (log cfu/g)	0.0799
Interlaboratory's standard deviation (log cfu/g)	0.1618
Reproducibility standard deviation (log cfu/g)	0.1805

### 3.1.9. *LISTERIA MONOCYTOGENES*

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

None significant effect of the analysis technique has been highlighted.

<i>Listeria monocytogenes</i>	
Assigned value of the contamination (log cfu/g)	3.127
Assigned value uncertainty (log cfu/g)	0.0090
Standard deviation for proficiency assessment (log cfu/g)	0.1040
Range of expected satisfactory values (log cfu/g)	[2.919 ; 3.335]
Standard deviation for precision (log cfu/g)	0.0736
Interlaboratory's standard deviation (log cfu/g)	0.0949
Reproducibility standard deviation (log cfu/g)	0.1201

## 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 unit n°3 was artificially contaminated.

279 laboratories obtained correct results.

6 laboratories obtained false positive results (respectively 2, 2, 2 and 3 false-positive for units n° 1, 2, 4 and 5).

3 laboratories obtained false negative results for unit n° 3.

### 3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

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

254 laboratories obtained correct results.

1 laboratory obtained false positive results for unit n° 4.

2 laboratories obtained false negative results (respectively 1 and 1 false-negative for units n°1 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 61<sup>th</sup> 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.