

## PROFICIENCY TEST « RAEMA »

### SCHEME N° 70 (9th MARCH 2020) GENERAL REPORT



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## 1. GENERAL DATA

### 1.1. PARTICIPATING LABORATORIES

343 laboratories participated to the 70<sup>th</sup> scheme. The sending was made on Monday 9th March 2020. We received 305 answers (88.9%). Due to the confinement period, this percentage is less important than usual. Considering the number of replies, this has no impact on the robustness of the statistical analysis.

### 1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+8	J0+15
Nb of laboratories	4	206	46	23	16	2	5	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  $10^3$  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  $5.10^2$  cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of  $2.10^2$  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 25 cfu/g in 2 units ;
- one strain of *Listeria monocytogenes* at a concentration level of  $2.10^3$  cfu/g in 4 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.

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

### 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 16, 23 and 30 March 2020. These checks were realized by a subcontractor accreditated by Cofrac.

### 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

**305** laboratories (100%) specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+14	J0+15	J0+16	J0+17	J0+22
Nb of laboratories	32	57	28	7	1	1	116	34	9	2	10	5	1	1	1

### 1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

**301** laboratories (98.7%) specified it. The average temperature is **4.0°C** with a standard deviation of 1.2°C. The given data 20 and 21°C given by 3 laboratories were not taken into account for this calculation.

## 2. EXPLOITATION OF ANALYSIS REPORT

### 2.1. PREPARATION OF THE INITIAL SUSPENSION

For **305** answers (100%) :

191 laboratories (62.6%) prepare the initial suspension with adding diluent to powder.  
114 laboratories (37.4%) prepare the initial suspension with adding powder to diluent.

### 2.2. DILUENT USED FOR THE INITIAL SUSPENSION

This data has been added to have all needed elements for this stage.

For **304** answers (99.7%) :

271 laboratories (88.9%) use Buffered Peptone Water for the initial suspension.  
33 laboratories (10.8%) used another diluent for the initial suspension.

### 2.3. HOMOGENEIZATION TECHNIQUE

For **304** answers (99.7%) :

290 laboratories (95.1%) homogenize their sampling with a Stomacher<sup>ND</sup>.  
14 laboratories (4.6%) used another technique (manual, magnétic or other).

### 2.4. RESUSCITATION'S CONDITIONS

#### 2.4.1. DURATION

**297** laboratories (97.4%) specified it.

The average duration is **27.2 min** with a standard deviation of 15.3 min. The data 120, 180 and 1440 min given by 6 laboratories was not taken into account for this calculation.

#### 2.4.2. TEMPERATURE

297 laboratories (97.4%) specified it.

The average temperature is **21.2°C** with a standard deviation of 3.4°C.

#### 2.5. MICROORGANISM AT 30°C

290 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF EN ISO 4833-1	180
	AFNOR 3M-01/1-09/89	45
	NM ISO 4833-1	18
	NF EN ISO 4833-2	15
	AFNOR BIO-12/35-05/13	11
	XP V08-034	4
	Other	17
	+ V08-100 (spiral)	14
<b>Culture medium</b>		
	Plate Count Agar	215
	Petrifilms	47
	Plate Count Agar + Milk	16
	Tempo AC	11
	Other	1
<b>Preparation</b>		
	Home made	106
	Ready to use not pre-poured	118
	Ready to use, plate, film, card	66
<b>Plating method</b>		
	Surface	61
	Pour	214
	Culture medium for card	12
<b>1<sup>st</sup> dilution retained</b>		
	- 1	13
	- 2	14
	- 3	239
	- 4	10
	- 5	1
	1/400	8
	1/4000	1
<b>Incubation temperature</b>		
	30°C	287
	37°C	2
<b>Incubation duration</b>		
	69-73 h	243
	40-48 h	42
	24-26 h	4

## 2.6.ENTEROBACTERIACEA

256 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF V08-054	107
	→ NM 08.0.109 <sup>(1)</sup>	22
	NF EN ISO 21528-2	63
	AFNOR 3M-01/6-09/97	41
	AFNOR BIO-12/21-12/06	9
	AFNOR AES-10/07-01/08	8
	AFNOR BRD-07/24-11/13	3
	Other	3
	+ V08-100 (spiral)	5
<b>Culture medium</b>		
	VRBG	189
	Petrifilms	43
	Tempo EB	9
	Rebecca	9
	Rapid'Enterobacteriaceae	3
	Other	2
<b>Preparation</b>		
	Home made	84
	Ready to use not pre-poured	117
	Ready to use, plate, film, card	55
<b>1<sup>st</sup> dilution retained</b>		
	- 1	188
	- 2	57
	1/40	1
	1/400	7
<b>Incubation temperature</b>		
	37±1°C	155
	30°C	92
	35°C	9
<b>Incubation duration</b>		
	18-24 h	251
	48 h	5
<b>Confirmatory test</b>	Yes	51
(New technical data requested to cover all stages of the standard)	No	199

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

## 2.7.TOTAL COLIFORMS

**216** laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF V08-050	109
	→ NM 08.0.142 <sup>(2)</sup>	10
	NF ISO 4832	53
	AFNOR 3M	20
	NM ISO 4832	13
	AFNOR BIO-12/17-12/05	4
	AFNOR BRD-07/08-12/04	3
	Other	4
	+ V08-100 (spiral)	3
<b>Culture medium</b>		
	VRBL	186
	Petrifilms	22
	Tempo TC	4
	Rapid Ecoli	3
	Other	1
<b>Preparation</b>		
	Home made	83
	Ready to use not pre-poured	107
	Ready to use, plate, film, card	25
<b>1<sup>st</sup> dilution retained</b>		
	-1	185
	-2	24
	1/40	1
	1/400	3
<b>Incubation temperature</b>		
	30°C	202
	37±1°C	14
<b>Incubation duration</b>		
	20-27 h	214
	48 h	1

AFNOR 3M method including :

3 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

197 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF V08-060	140
	→ NM 08.0.124 <sup>(3)</sup>	26
	AFNOR 3M	20
	NF ISO 4832	8
	Other	3
	+ V08-100 (spiral)	1
<b>Culture medium</b>		
	VRBL	175
	Petrifilms	21
	Other	1
<b>Preparation</b>		
	Home made	77
	Ready to use not pre-poured	99
	Ready to use, plate, film, card	21
<b>1<sup>st</sup> dilution retained</b>		
	-1	176
	-2	19
<b>Incubation temperature</b>		
	42-45°C	194
	37°C	2
	30°C	1
<b>Incubation duration</b>		
	20-24 h	196
	48 h	1

AFNOR 3M method including :

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

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

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

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

## 2.9.ESCHERICHIA COLI

271 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF ISO 16649-2	155
	AFNOR 3M	39
	NM ISO 16649-2	21
	AFNOR BRD-07/01-07/93	14
	AFNOR BIO-12/13-02/05	10
	AFNOR AES-10/06-01/08	9
	NF EN ISO 16649-3	5
	AFNOR BIO-12/05-01/99	3
	Other	15
	+ V08-100 (spiral)	4
<b>Culture medium</b>		
	TBX	183
	Petrifilms	41
	Rapid E. coli	19
	Rebecca	11
	Tempo EC	10
	Coli ID	6
	Other	1
<b>Preparation</b>		
	Home made	87
	Ready to use not pre-poured	132
	Ready to use, plate, film, card	52
<b>Plating method</b>		
	Surface	39
	Pour	217
	Culture medium for card	13
<b>1<sup>st</sup> dilution retained</b>		
	-1	242
	-2	16
	1/40	3
	1/400	6
<b>Incubation temperature</b>		
	41-46°C	236
	37±1°C	34
	30°C	1
<b>Incubation duration</b>		
	16-25 h	267
	44-48 h	4

AFNOR 3M method including :

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

## 2.10. ANAEROBIC SULFITE-REDUCING BACTERIA

217 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF V08-061	140
	→ NM 08.0.154 <sup>(4)</sup>	3
	→ NM 08.0.125 <sup>(4)</sup>	11
	NF ISO 15213	39
	NM ISO 15213	15
	Other	9
<b>Culture medium</b>		
	TSC	203
	Iron Sulfite agar	6
	TSN	5
	Other	3
<b>Preparation</b>		
	Home made	87
	Ready to use not pre-poured	109
	Ready to use, plate, film, card	21
<b>Seeding way</b> (New technical data requested to take into account different seeding ways)	Plates	135
	Tubes	80
<b>1<sup>st</sup> dilution retained</b>	-1	182
	-2	33
<b>Incubation temperature</b>	44-49°C	151
	37°C	65
	30°C	1
<b>Incubation duration</b>	18-24 h	180
	48 h	31
	72 h	4
	12-16 h	2

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

## 2.11. CLOSTRIDIUM PERFRINGENS

169 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>	NF EN ISO 7937	136
	NM ISO 7937	19
	Other	14
<b>Culture medium</b>	TSC	167
	Other	2
<b>Preparation</b>	Home made	59
	Ready to use not pre-poured	105
	Ready to use, plate, film, card	5
<b>1<sup>st</sup> dilution retained</b>	-1	158
	-2	8
<b>Incubation temperature</b>	37°C	158
	44-46°C	11
<b>Incubation duration</b>	18-24 h	163
	48 h	5
	72 h	1
<b>Confirmation test</b>	None	30
	Lactose-sulfite	126
	Strip	5
	Other	4

## 2.12. COAGULASE POSITIVE STAPHYLOCOCCI

270 laboratories performed the enumeration.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	NF EN ISO 6888-2	120
	NF V 08-057-1	40
	→ NM 08.0.112 <sup>(5)</sup>	5
	NF EN ISO 6888-1	36
	NM ISO 6888-1	18
	AFNOR 3M-01/9-04/03	15
	AFNOR BKR-23/10-12/15	11
	AFNOR BIO-12/28-04/10	10
	NordVal No :049	4
	NM ISO 6888-2	4
	Other	7
	+ V08-100 (spiral)	4
<b>Culture medium</b>		
	RPF	124
	BP+egg yolk tellurite	81
	BP+egg yolk tellurite+ sulfamethazine	16
	Petrifilm	16
	Easy Staph	16
	Tempo STA	10
	Rapid Staph	5
	Other	2
<b>Preparation</b>		
	Home made	63
	Ready to use not pre-poured	110
	Ready to use, plate, film, cards	97
<b>Plating method</b>		
	Surface	138
	Pour	119
	Culture medium for card	11
<b>1<sup>st</sup> dilution retained</b>		
	-1	105
	-2	149
	-3	4
	1/40	5
	1/400	3
<b>Incubation temperature</b>		
	37±1°C	266
	30°C	4
<b>Incubation duration</b>		
	42-48 h	191
	18-25 h	78
	72 h	1
<b>Confirmation test</b>		
	None	161
	Staphylo-coagulase	82
	Clumping factor	7
	DNase	11
	Other	7

<sup>(5)</sup> Similar method to NF V 08-057-1 according to ONSSA.

## 2.13. LISTERIA MONOCYTOGENES – ENUMERATION

212 laboratories performed the enumeration.

### RESUSCITATION

77 laboratories announce the realization of a resuscitation step.

The average duration for these laboratories is **46.3 min** with a standard deviation of 24.9 min.

The average temperature for these laboratories is **21.2°C** with a standard deviation of 3.6°C.

Parameters	Mode	Nb laboratories
<b>Method</b>		
	AFNOR AES-10/05-09/06	61
	NF EN ISO 11290-2	57
	AFNOR BKR-23/05-12/07	45
	NM ISO 11290-2	18
	AFNOR BRD-07/05-09/01	17
	AFNOR BRD-07/17-01/09	6
	Other	8
<b>Resuscitation step</b>	Yes	77
	No	121
<b>Resuscitation medium</b>	Buffered Peptone Water	60
	Fraser base	12
	Other	3
<b>Enumeration medium</b>	ALOA Count	101
	Compass Listeria	66
	Rapid Lmono	21
	AL Agar	12
	OCLA	5
	Palcam	4
	Other	2
<b>Preparation</b>	Home made	30
	Ready to use not pre-poured	46
	Ready to use, plate, film, card	135
<b>Plating method</b>	Surface	173
	Pour	37
	Culture medium for card	0

Parameters	Mode	Nb laboratories
<b>1<sup>st</sup> dilution retained</b>	-1	139
	-2	69
	-6	1
<b>Incubation temperature</b>	37°C	210
	30°C	2
<b>Incubation duration</b>	46-51 h	176
	22-24 h	36
<b>Confirmation test</b>	None	45
	Biochemical	128
	Biochemical + CAMP	29
	Other	8
<b>Nb of colonies tested per plate</b>	1	48
	2-3	16
	5	87
	10	1
	18	1
	100	1
	150	2

## 2.14. SALMONELLA – DETECTION

**275** laboratories performed the detection.

Methods used by laboratories are clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>		
	NF EN ISO 6579-1	73
	AFNOR BKR 23/07-10/11 (IRIS Salmonella)	64
	AFNOR BRD 07/11-12/05 (Rapid Salmonella)	33
	NM ISO 6579-1	28
	AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella)	21
	AFNOR BIO 12/32-10/11 (VIDAS SPT)	20
	AFNOR BIO 12/41-03/17 (SALMA One day)	18
	Other	18

No detail of methodology was asked to laboratories using other methods than 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

The detail of the methodology followed by 101 laboratories using NF EN ISO 6579-1 and NM ISO 6579-1 method, and the 18 laboratories using another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>		
	NF EN ISO 6579-1	73
	NM ISO 6579-1	28
	Other	18
<b>Pre-enrichment medium</b>	Buffered Peptone Water	113
	Other	5
<b>Pre-enrichment temperature</b>	36-37°C	111
	41-42.5°C	5
	20-22°C	2
<b>Pre-enrichment duration</b>	16-20 h	81
	22-26 h	37
<b>Enrichment medium</b>	RVS	103
	MKTTn	95
	Selenite-cystine broth	19
	Other	5
<b>Isolation medium</b>	XLD	94
	Hektoen	34
	Bismuth Sulfate	18
	ASAP	11
	Brilliance Salmonella	10
	IRIS Salmonella agar	10
	GVB	10
	SS	7
	Compass Salmonella	5
	Rambach	5
	Rapid Salmonella	3
	Other	11
<b>Confirmation test</b>	Biochemical	49
	Biochemical + serological agglutination	60
	Other	7

## 2.15. LISTERIA MONOCYTOGENES – DETECTION

237 laboratories performed the detection.

Parameter	Mode	Nb laboratories
<b>Method</b>		
AFNOR AES 10/03-09/00 (ALOA one day)		61
AFNOR BKR 23/02-11/02 (Compass L. mono)		50
NF EN ISO 11290-1		44
NM ISO 11290-1		24
AFNOR BRD 07/04-09/98 (Rapid' L. mono)		21
AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C)		8
AFNOR BIO 12/27-02/10 (VIDAS LMX)		7
AFNOR BRD 07/16-01/09 (Agar Listeria)		6
AFNOR BIO 12/02-06/94 (VIDAS Listeria)		2
AFNOR UNI 03/04-04/05 (Listeria Precis)		2
Other		12

No detail of methodology was asked to laboratories using other method than 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	ChromID 37°C - 24h
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

The detail of the methodology followed by 68 laboratories using NF EN ISO 11290-1 and NM ISO 11290-1 methods, and the 12 laboratories using another method, is clarified in the following table :

Parameter	Mode	Nb laboratories
<b>Method</b>		
	NF EN ISO 11290-1	44
	NM ISO 11290-1	24
	Other	12
<b>Primary enrichment medium</b>	Half-Fraser	66
	One broth Listeria	3
	Other	10
<b>Primary enrichment temperature</b>	30°C	71
	37°C	8
<b>Primary enrichment duration</b>	20-28 h	77
	48 h	1
<b>Secondary enrichment medium</b>	Fraser	64
	Other	3
<b>Secondary enrichment temperature</b>	37±1°C	63
	30°C	4
	24°C	1
<b>Secondary enrichment duration</b>	22-25 h	54
	46-48 h	14
<b>Isolation medium</b>	Palcam	48
	Ottaviani et Agosti	39
	Compass Listeria	28
	Oxford	12
	Rapid L'mono	4
	Brilliance Listeria	3
	Other	1
<b>Isolation temperature</b>	37°C	74
	30°C	1
<b>Isolation duration</b>	46-48 h	50
	24 h	25
<b>Confirmation test</b>	None	5
	Biochemical	47
	Biochemical + CAMP	24
	Other	1
<b>Nb of colonies per plate</b>	1	25
	2-4	9
	5	31
	6	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 value 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 a 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 laboratory'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)	4.876
Assigned value uncertainty (log CFU/g)	0.0066
Standard deviation for proficiency assessment (log CFU/g)	0.0882
Standard deviation for precision (log CFU/g)	0.0580
Interlaboratory's standard deviation (log CFU/g)	0.0843
Reproducibility standard deviation (log CFU/g)	0.1023

### 3.1.2. ENTEROBACTERIACEAE

A significant “effect” of the culture media, 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 :

Enterobacteriaceae	Group 1	Group 2	Group 3
Assigned value of the contamination (log CFU/g)	2.829	2.996	3.169
Assigned value uncertainty (log CFU/g)	0.0184	0.0358	0.0327
Standard deviation for proficiency assessment (log CFU/g)	0.1968	0.1765	0.1382
Standard deviation for precision (log CFU/g)		0.0897	
Interlaboratory's standard deviation (log CFU/g)	0.1927	0.1719	0.1323
Reproducibility standard deviation (log CFU/g)	0.2125	0.1939	0.1598

### 3.1.3. TOTAL COLIFORMS

A significant “effect” of the culture media and manufacturer 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)	2.790
Assigned value uncertainty (log CFU/g)	0.0198
Standard deviation for proficiency assessment (log CFU/g)	0.2270
Standard deviation for precision (log CFU/g)	0.0857
Interlaboratory's standard deviation (log CFU/g)	0.2238
Reproducibility standard deviation (log CFU/g)	0.2396

### 3.1.4. THERMOTOLERANT COLIFORMS

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 :

Thermotolerant coliforms	
Assigned value of the contamination (log CFU/g)	2.759
Assigned value uncertainty (log CFU/g)	0.0182
Standard deviation for proficiency assessment (log CFU/g)	0.1987
Standard deviation for precision (log CFU/g)	0.0851
Interlaboratory's standard deviation (log CFU/g)	0.1950
Reproducibility standard deviation (log CFU/g)	0.2127

### 3.1.5. *ESCHERICHIA COLI*

None significant effect of the analysis technique has been highlighted.

<i>Escherichia coli</i>	
Assigned value of the contamination (log CFU/g)	2.719
Assigned value uncertainty (log CFU/g)	0.0154
Standard deviation for proficiency assessment (log CFU/g)	0.1992
Standard deviation for precision (log CFU/g)	0.0920
Interlaboratory's standard deviation (log CFU/g)	0.1949
Reproducibility standard deviation (log CFU/g)	0.2155

### 3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

Only units n°4 and 5 were artificially contaminated.

A significant “effect” of the culture media 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)	2.321
Assigned value uncertainty (log CFU/g)	0.0183
Standard deviation for proficiency assessment (log CFU/g)	0.2092
Standard deviation for precision (log CFU/g)	0.1037
Interlaboratory's standard deviation (log CFU/g)	0.1960
Reproducibility standard deviation (log CFU/g)	0.2217

Comment :

- 9 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 10 to 1900 CFU/g.
- 7 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 20 to 1600 CFU/g.
- 11 laboratories detected ASR in unit n°3 non-artificially contaminated by *C. perfringens* with a contamination level from 2 to 1500 CFU/g.

### 3.1.7. *CLOSTRIDIUM PERFRINGENS*

Only units n°4 and 5 were artificially contaminated.

A significant “effect” of the culture media 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)	2.300
Assigned value uncertainty (log CFU/g)	0.0189
Standard deviation for proficiency assessment (log CFU/g)	0.1902
Standard deviation for precision (log CFU/g)	0.0985
Interlaboratory's standard deviation (log CFU/g)	0.1770
Reproducibility standard deviation (log CFU/g)	0.2026

Comment :

- 4 laboratories detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 100 to 1500 CFU/g.
- 4 laboratories detected *C. perfringens* in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 10 to 1400 CFU/g.
- 5 laboratories detected *C. perfringens* in unit n°3 non-artificially contaminated by *C. perfringens* with a contamination level from 50 to 1400 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)	3.441
Assigned value uncertainty (log CFU/g)	0.0124
Standard deviation for proficiency assessment (log CFU/g)	0.1594
Standard deviation for precision (log CFU/g)	0.0823
Interlaboratory's standard deviation (log CFU/g)	0.1551
Reproducibility standard deviation (log CFU/g)	0.1756

### 3.1.9. *LISTERIA MONOCYTOGENES*

Only units n°2, 3, 4 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)	3.367
Assigned value uncertainty (log CFU/g)	0.0097
Standard deviation for proficiency assessment (log CFU/g)	0.1098
Standard deviation for precision (log CFU/g)	0.0661
Interlaboratory's standard deviation (log CFU/g)	0.1047
Reproducibility standard deviation (log CFU/g)	0.1238

### 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°4 and 5 were artificially contaminated.

261 laboratories obtained correct results.

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

11 laboratories obtained false negative results (respectively 4 and 9 false-negative for units n°4 and 5).

#### 3.2.2. DETECTION – *LISTERIA MONOCYTOGENES*

Only units n°2, 3, 4 and 5 were artificially contaminated.

234 laboratories obtained correct results.

2 laboratories obtained false positive results for unit n°1.

3 laboratories obtained false negative results (respectively 1, 0, 0 and 2 false-negative for units n°2, 3, 4 and 5).

### 3.3.EVOLUTION OF PERFORMANCE

You will find, on each page of your performance's assessment, a graph representing evolution of it on different tests since the 50<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.