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PROFICIENCY TEST « RAEMA »

SCHEME N° 71 (5th OCTOBER 2020)

GENERAL REPORT



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1. GENERAL DATA

1.1.PARTICIPATING LABORATORIES

345 laboratories participated to the 71th scheme. The sending was made on Monday 5th October 2020. We received **341** answers (98.8%).

1.2.DELIVERY TIME OF THE PARCEL

| Reception | JO | J0+1 | J0+2 | J0+3 | J0+4 | J0+5 | J0+7 | J0+8 | J0+9 | J0+11 | J0+14 |
|-----------------------|----|------|------|------|------|------|------|------|------|-------|-------|
| Nb of laboratories | 3 | 204 | 80 | 24 | 12 | 1 | 6 | 2 | 3 | 3 | 3 |

1.3.INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

The sample included :

- one strain of *Enterococcus sp.* at a concentration level of 10⁵ cfu/g in 5 units ;
- one strain of *Citrobacter sp.* at a concentration level of 10³ cfu/g in 5 units ;
- one strain of Serratia liquefaciens at a concentration level of 5.10² cfu/g in 5 units ;
- one strain of *Escherichia coli* at a concentration level of 10² cfu/g in 5 units ;
- one strain of *Clostridium perfringens* at a concentration level of 10² cfu/g in 3 units ;
- one strain of *Staphylococcus aureus* at a concentration level of 4.10³ cfu/g in 5 units ;
- one strain of Salmonella Anatum at a concentration level of 25 cfu/g in 3 units ;
- one strain of *Listeria monocytogenes* at a concentration level of 2.10³ cfu/g in 2 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⁾Coordinator of the proficiency test « RAEMA »



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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 12, 19 and 26 October 2020. These checks were realized by a subcontractor accreditated 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

341 laboratories (100%) specified it.

| Analysis time | JO | J0+1 | J0+2 | J0+3 | J0+4 | J0+5 | J0+7 | J0+8 | J0+9 | J0+11 | J0+14 | J0+15 | J0+16 |
|-----------------------|----|------|------|------|------|------|------|------|------|-------|-------|-------|-------|
| Nb of laboratories | 1 | 28 | 42 | 44 | 10 | 2 | 138 | 48 | 9 | 1 | 13 | 4 | 1 |

1.4.2. PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

337 laboratories (98.8%) specified it. The average temperature is **3.9°C** with a standard deviation of 0.8°C. The given data 22, 30 and 44°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 **341** answers (100%) :

213 laboratories (62.5%) prepare the initial suspension with adding diluent to powder. 128 laboratories (37.5%) prepare the initial suspension with adding powder to diluent.

2.2.DILUENT USED FOR THE INITIAL SUSPENSION

For **340** answers (99.7%) :

282 laboratories (82.7%) use Buffered Peptone Water for the initial suspension.

48 laboratories (14.1%) use Peptone salt for the initial suspension.

10 laboratories (2.9%) used another diluent for the initial suspension.

2.3.HOMOGENEIZATION TECHNIQUE

For **341** answers (100%) : 314 laboratories (92.1%) homogeneize their sampling with a StomacherND. 27 laboratories (7.9%) used another technique (manual, magnétic or other).

2.4.RESUSCITATION'S CONDITIONS

2.4.1. DURATION

324 laboratories (95%) specified it.

The average duration is **26.8 min** with a standard deviation of 13.9 min. The data 90, 120 and 1440 min given by 3 laboratories was not taken into account for this calculation.



2.4.2. TEMPERATURE

324 laboratories (95.0%) specified it. The average temperature is **21.4°C** with a standard deviation of 3.0°C.

2.5.MICROORGANISM AT 30°C

319 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|--|--|
| Method | NF EN ISO 4833-1 AFNOR 3M-01/1-09/89 NF EN ISO 4833-2 NM ISO 4833-1 AFNOR BIO-12/35-05/13 XP V08-034 Other + V08-100 (spiral) | 202 52 16 15 11 6 17 |
| Culture medium | Plate Count Agar Petrifilms Plate Count Agar + Milk Tempo AC Other | 239 52 16 11 0 |
| Preparation | Home made Ready to use not pre-poured Ready to use, plate, film, card | 111 134 72 |
| Plating method | Surface Pour Culture medium for card | 73 225 11 |
| 1 st dilution retained | - 1 - 2 - 3 - 4 - 5 1/400 1/4000 | 10 20 271 6 2 5 4 |
| Incubation temperature | 30°C 37°C | 316 1 |
| Incubation duration | 68-73 h 40-48 h 24-26 h | 262 51 2 |

71th scheme (edition 04/12/20)

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2.6.ENTEROBACTERIACEA

286 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|------------------------|--|-----------------|
| Method | NF V08-054 | 113 |
| | $\rightarrow NM \ 08.0.109^{(1)}$ | 24 |
| | NF EN ISU 21528-2 | 70 |
| | AFNOR 3M-01/6-09/97 | 48 10 |
| | AFNOR DIO-12/21-12/00 AFNOR AFS_10/07_01/08 | 10 Q |
| | AFNOR BRD-07/24-11/13 | 9 6 |
| | Other | 6 |
| | + V08-100 (spiral) | 1 |
| Culture medium | VRBG | 210 |
| | Petrifilms | 50 |
| | Tempo EB | 10 |
| | Rebecca | 9 |
| | Rapid'Enterobacteriaceae | 6 |
| | Other | 0 |
| Preparation | Home made | 91 |
| - | Ready to use not pre-poured | 132 |
| | Ready to use, plate, film, card | 61 |
| | 4 | 100 |
| 1 dilution retained | - | 192 |
| | - 2 | <u></u> उ |
| | 1/40 | 2 |
| | 1/400 | 7 |
| Incubation temperature | 37±1°C | 181 |
| - | 30°C | 92 |
| | 35±1°C | 11 |
| Incubation duration | 20-27 h | 274 |
| | 48 h | 8 |
| | 2-4 h | 2 |
| Confirmatory | Yee | F7 |
| Commutatory test | No | ں ت 222 |
| | 110 | |

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



2.7.TOTAL COLIFORMS

240 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|---|--|
| Method | NF V08-050 → NM 08.0.142 ⁽²⁾ NF ISO 4832 AFNOR 3M NM ISO 4832 AFNOR BIO-12/17-12/05 AFNOR BRD-07/08-12/04 Other + V08-100 (spiral) | 119 12 52 25 16 7 4 5 |
| Culture medium | VRBL Petrifilms Tempo TC Rapid Ecoli Other | 199 26 7 5 3 |
| Preparation | Home made Ready to use not pre-poured Ready to use, plate, film, card | 86 120 33 |
| 1 st dilution retained | -1 -2 -3 1/40 1/400 | 195 37 1 2 3 |
| Incubation temperature | 30°C 35-37°C | 220 19 |
| Incubation duration | 20-26 h 48 h 96 h | 236 2 1 |

AFNOR 3M method including :

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

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

2 laboratories specified utilization of AFNOR 3M Petrifilm CC method.

⁽²⁾ Similar method to NF V 08-050 according to ONSSA.



2.8.THERMOTOLERANT COLIFORMS

214 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|--|-----------------|
| Method | NF V08-060 | 146 |
| | \rightarrow INIVI UO.U. 124 $^{\circ}$ | 21 |
| | | 20 |
| | NF ISO 4032 Other | 9 |
| | | 0 4 |
| | + voo-100 (spiral) | 0 |
| Culture medium | VRBI | 183 |
| Callaro moulam | Petrifilms | 29 |
| | Other | 2 |
| | | _ |
| Preparation | Home made | 80 |
| - | Ready to use not pre-poured | 106 |
| | Ready to use, plate, film, card | 27 |
| | | |
| 1 st dilution retained | -1 | 180 |
| i dilution retained | -2 | .32 |
| | L | 02 |
| Incubation temperature | 42-45°C | 211 |
| ····· | 35-37°C | 2 |
| | 30°C | 1 |
| | | |
| Incubation duration | 20-26 h | 210 |
| | 48 h | 3 |
| | 96 h | 1 |

AFNOR 3M method including :

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

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

2 laboratories specified utilization of AFNOR 3M Petrifilm CC method.

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

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



2.9.ESCHERICHIA COLI

300 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|--|-----------------|
| Method | NF ISO 16649-2 | 172 |
| | AFNOR 3M | 44 |
| | NM ISU 16649-2 | 22 |
| | AFNOR BRD-07/01-07/93 | 17 |
| | AFNOR BIO-12/13-02/05 AFNOR AFS 10/06 01/08 | 11 8 |
| | ΔENIOR BIO-12/05-01/00 | 0 |
| | NE EN ISO 16649-3 | 1 |
| | Other | 17 |
| | + V08-100 (spiral) | 1 |
| Culture medium | ТВХ | 201 |
| | Petrifilms | 46 |
| | Rapid E. coli | 22 |
| | Tempo EC | 11 |
| | | 9 |
| | Othor | 0 1 |
| | Other | I |
| Preparation | Home made | 89 |
| | Ready to use not pre-poured | 153 |
| | Ready to use, plate, film, card | 55 |
| Plating method | Surface | 45 |
| - | Pour | 236 |
| | Culture medium for card | 12 |
| 1 st dilution retained | -1 | 282 |
| | -2 | 7 |
| | _ 1/40 | 3 |
| | 1/400 | 6 |
| Incubation temperature | 41-45°C | 263 |
| | 35-37°C | 34 |
| | 30°C | 1 |
| Incubation duration | 18-28 h | 293 |
| | 48 h | 4 |
| | 216 | 1 |

AFNOR 3M method including :

14 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.



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2.10. ANAEROBIC SULFITE-REDUCING BACTERIA

242 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|---|----------------------------------|
| Method | NF V08-061 $\rightarrow NM \ 08.0.154^{(4)}$ $\rightarrow NM \ 08.0.125^{(4)}$ NF ISO 15213 NM ISO 15213 Other | 161 5 12 42 10 12 |
| Culture medium | TSC Iron Sulfite agar TSN Other | 221 6 7 5 |
| Preparation | Home made Ready to use not pre-poured Ready to use, plate, film, card | 96 116 30 |
| Seeding way | Plates Tubes | 150 89 |
| 1 st dilution retained | -1 -2 | 205 36 |
| Incubation temperature | 44-48°C 37°C | 176 66 |
| Incubation duration | 18-24 h 44-48 h 72 h 12-16 h | 201 30 8 3 |

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



2.11. CLOSTRIDIUM PERFRINGENS

195 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|---------------------------------|-----------------|
| Method | NF EN ISO 7937 | 155 |
| | NM ISO 7937 | 22 |
| | Other | 18 |
| Culture medium | TSC | 189 |
| | Other | 3 |
| Preparation | Home made | 68 |
| roparation | Ready to use not pre-poured | 119 |
| | Ready to use, plate, film, card | 6 |
| | | |
| 1 st dilution retained | -1 | 182 |
| | -2 | 12 |
| Incubation temperature | 37±1°C | 183 |
| · | 44-46°C | 10 |
| | 34°C | 1 |
| Incubation duration | 18-24 h | 188 |
| | 48 h | 4 |
| | 72 h | 2 |
| Confirmation test | None | 35 |
| | Lactose-sulfite | 141 |
| | Strip | 8 |
| | Other | 6 |



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2.12. COAGULASE POSITIVE STAPHYLOCOCCI

298 laboratories performed the enumeration.

| Parameters | Mode | Nb laboratories |
|-----------------------------------|--|-----------------|
| Method | NF EN ISO 6888-2 | 141 |
| | NF EN ISO 6888-1 | 61 |
| | NM ISO 6888-1 | 18 |
| | AFNOR 314-01/9-04/03 | 17 |
| | AFNOR BRR-23/10-12/13 AENOR BIO 12/28 04/10 | 10 |
| | Nord\/al No :049 | 10 |
| | NM ISO 6888-2 | 4 |
| | Other | 26 |
| | + V08-100 (spiral) | 1 |
| Culture medium | RPF | 139 |
| | BP+egg yolk tellurite | 81 |
| | Petrifilm | 18 |
| | Easy Staph | 18 |
| | BP+egg yolk tellurite+ sulfamethazine | 1/ |
| | Tempo STA Rapid Stanb | 13 |
| | Other | 4 4 |
| Prenaration | Home made | 69 |
| rieparation | Ready to use not pre-poured | 131 |
| | Ready to use, plate, film, cards | 96 |
| Plating method | Surface | 147 |
| | Pour | 137 |
| | Culture medium for card | 11 |
| 1 st dilution retained | -1 | 110 |
| | -2 | 174 |
| | -3 | 4 |
| | 1/40 | 3 |
| | 1/400 | 5 |
| Incubation temperature | 36-37°C | 295 |
| | 30°C | 1 |
| Incubation duration | 42-48 h | 206 |
| | 18-25 h | 86 |
| | 72 h | 2 |
| | 34 h | 1 |
| | 90 (1 | 1 |
| Confirmation test | None | 182 |
| | Staphylo-coagulase | 83 |
| | Clumping factor | 9 |
| | DNase | 11 |
| | Other | 6 |



2.13. LISTERIA MONOCYTOGENES – ENUMERATION

239 laboratories performed the enumeration.

RESUSCITATION

67 laboratories announce the realization of a resuscitation step.

The average duration for these laboratories is **41.1 min** with a standard deviation of 19.2 min (The data 1440 min given by 1 laboratory was not taken into account for this calculation).

The average temperature for these laboratories is **21.1°C** with a standard deviation of 2.8°C.

| Parameters | Mode | Nb laboratories |
|----------------------|--|--------------------------------------|
| Method | NF EN ISO 11290-2 AFNOR AES-10/05-09/06 AFNOR BKR-23/05-12/07 AFNOR BRD-07/05-09/01 NM ISO 11290-2 AFNOR BRD-07/17-01/09 Other | 72 65 43 24 21 5 8 |
| Resuscitation step | Yes No | 67 159 |
| Resuscitation medium | Buffered Peptone Water Fraser base Other | 56 7 1 |
| Enumeration medium | ALOA Count Compass Listeria Rapid Lmono AL Agar OCLA Palcam Other | 116 73 26 12 5 3 3 |
| Preparation | Home made Ready to use not pre-poured Ready to use, plate, film, card | 27 51 159 |
| Plating method | Surface Pour Culture medium for card | 198 36 0 |



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| Parameters | Mode | Nb laboratories |
|-----------------------------------|--------------------|-----------------|
| 1 st dilution retained | -1 | 174 |
| | -2 | 64 |
| Incubation temperature | 37°C | 234 |
| | 30°C | 4 |
| Incubation duration | 40-48 h | 193 |
| | 22-27 h | 45 |
| Confirmation test | None | 44 |
| | Biochemical | 144 |
| | Biochemical + CAMP | 37 |
| | Other | 6 |
| Nb of colonies tested per | 1 | 66 |
| plate | 2-4 | 19 |
| • | 5 | 94 |
| | 6 | 1 |



2.14. SALMONELLA – DETECTION

307 laboratories performed the detection. Methods used by laboratories are clarified in the following table :

| Parameter | Mode | Nb laboratories |
|-----------|---|-----------------|
| Method | NF EN ISO 6579-1 | 94 |
| | AFNOR BKR 23/07-10/11 (IRIS Salmonella) | 68 |
| | AFNOR BRD 07/11-12/05 (Rapid Salmonella) | 28 |
| | NM ISO 6579-1 | 27 |
| | AFNOR BIO 12/41-03/17 (SALMA One day) | 27 |
| | AFNOR BIO 12/16-09/05 (VIDAS Easy Salmonella) | 25 |
| | AFNOR BIO 12/32-10/11 (VIDAS SPT) | 24 |
| | Other | 14 |

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 VIDAS Easy Salmonella | BPW / 37°C - 16/20h | SX2 / 41,5°C - 22/26h | Chrom ID / 37°C - 24h |
| AFNOR BIO 12/32-10/11 VIDAS SPT | | BPW + Salmonella supplement / 41,5°C - 18/24h | Chrom ID / 37°C - 24h |
| AFNOR BKR 23/07-10/11 IRIS Salmonella | | IRIS Salmonella Enrichment / 41,5°C - 18±2h | IRIS / 37°C - 24±3h |
| AFNOR BRD 07/11-12/05 Rapid Salmonella | | BPW + Salmonella capsule / 41,5°C - 18±2h | Rapid Salmonella / 37°C - 24±2h |
| AFNOR BIO 12/41-03/17 SALMA One day | | BPW + Salmonella supplement / 41.5°C – 16/24h | SALMA / 37°C - 24±3h |



The detail of the methodology followed by 121 laboratories using NF EN ISO 6579-1 and NM ISO 6579-1 method, and the 14 laboratories using another method, is clarified in the following table :

| Parameter | Mode | Nb laboratories |
|----------------------------|---|--|
| Method | NF EN ISO 6579-1 NM ISO 6579-1 Other | 94 27 14 |
| Pre-enrichment medium | Buffered Peptone Water Other | 128 6 |
| Pre-enrichment temperature | 37±1°C 41-42.5°C 20-23°C | 121 8 4 |
| Pre-enrichment duration | 16-20 h 22-24 h | 93 40 |
| Enrichment medium | RVS MKTTn Selenite-cystine broth Other | 115 111 25 7 |
| Isolation medium | XLD Hektoen Bismuth Sulfate Rapid Salmonella IRIS Salmonella agar ASAP Brilliance Salmonella GVB SS Compass Salmonella Rambach Other | 101 37 20 17 14 13 9 9 9 9 5 1 9 |
| Confirmation test | Biochemical Biochemical + serological agglutination Other | 52 69 7 |



2.15. LISTERIA MONOCYTOGENES – DETECTION

272 laboratories performed the detection.

| Parameter | Mode | Nb laboratories |
|-----------|---|-----------------|
| Method | NF EN ISO 11290-1 | 67 |
| | AFNOR AES 10/03-09/00 (ALOA one day) | 59 |
| | AFNOR BKR 23/02-11/02 (Compass L. mono) | 50 |
| | AFNOR BRD 07/04-09/98 (Rapid' L. mono) | 25 |
| | NM ISO 11290-1 | 22 |
| | AFNOR BIO 12/11-03/04 (VIDAS LMO2-37°C) | 13 |
| | AFNOR BIO 12/27-02/10 (VIDAS LMX) | 8 |
| | AFNOR BIO 12/02-06/94 (VIDAS Listeria) | 5 |
| | AFNOR BRD 07/16-01/09 (Agar Listeria) | 4 |
| | AFNOR UNI 03/04-04/05 (Listeria Precis) | 3 |
| | AFNOR BIO 12/18-03/06 (VIDAS LDUO) | 3 |
| | AFNOR BIO 12/40-11/16 (GENE UP LMO) | 2 |
| | Other | 11 |

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.

| Máthad | Primar | Primary enrichment Secondary enrichme | | ondary enrichment | Isolation | |
|--|-----------------------|---------------------------------------|--------|-------------------|---|--|
| Method | Medium | Incubation | Medium | Incubation | isolation | |
| AFNOR BRD 07/04-09/98 Rapid' L. mono | Fraser 1/2 | 30°C - 24±2h | | | Rapid L'mono 37°C – 24h | |
| AFNOR BIO 12/02-06/94 VIDAS Listeria | Fraser 1/2 | 37°C - 26/30h | Fraser | 30°C - 24/26h | Palcam et Oxford 37°C – 24h | |
| AFNOR BIO 12/27-02/10 VIDAS LMX | LMX | 37°C - 26/30h | | | ChromID 37°C – 24h | |
| AFNOR BIO 12/11-03/04 VIDAS LMO2 (37°C) | Fraser 1/2 | 30°C - 24/26h | Fraser | 37°C - 24/26h | Chromogenic medium / Palcam / Oxford | |
| AFNOR AES 10/03-09/00 ALOA one day | Fraser 1/2 | 30°C - 24±2h | | | ALOA One Day 37°C – 24/48h | |
| AFNOR BKR 23/02-11/02 Compass L. mono | Fraser 1/2 | 30°C - 24±2h | | | Compass Listeria Agar 37°C – 24h | |
| AFNOR BRD 07/16-01/09 Agar Listeria | Fraser 1/2 | 30°C - 24±2h | | | Agar Listeria 37°C – 24h | |
| AFNOR UNI 03/04-04/05 Listeria Precis | One Broth Listeria | 30°C - 24±2h | | | Brilliance Listeria 37°C – 24h | |
| AFNOR BIO 12/40-11/16 GENE UP LMO | LPT | 35-37°C - 24±2h | | | ALOA 35-37°C – 24/48h | |
| AFNOR BIO 12/18-03/06 VIDAS LDUO | LX | 30°C - 24±2h | LX | 30°C - 24/26h | Chromogenic medium / Palcam / Oxford | |



The detail of the methodology followed by 89 laboratories using NF EN ISO 11290-1 and NM ISO 11290-1 methods, and the 11 laboratories using another method, is clarified in the following table :

| Parameter | Mode | Nb laboratories |
|-------------------------------------|---|-------------------------------------|
| Method | NF EN ISO 11290-1 NM ISO 11290-1 Other | 67 22 11 |
| Primary enrichment medium | Half-Fraser One broth Listeria Other | 89 2 8 |
| Primary enrichment temperature | 30°C 37°C 22°C | 92 5 1 |
| Primary enrichment duration | 23-27 h 48 h 1 h | 96 1 1 |
| Secondary enrichment medium | Fraser Other | 88 1 |
| Secondary enrichment temperature | 37±1°C 30°C 22°C | 85 2 1 |
| Secondary enrichment duration | 20-27 h 48 h 1 h | 69 18 1 |
| Isolation medium | Palcam Ottaviani et Agosti Compass Listeria Oxford Rapid L'mono Brilliance Listeria Other | 65 53 32 15 7 2 1 |
| Isolation temperature | 37°C 30°C | 96 1 |
| Isolation duration | 47-48 h 22-24 h | 57 39 |
| Confirmation test | None Biochemical Biochemical + CAMP Other | 8 59 26 4 |
| Nb of colonies per plate | 1 2-3 5 6 | 27 7 45 1 |

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3. ASSESSMENT OF PERFORMANCE (INDIVIDUEL REPORTS)

3.1.PERFORMANCES IN ENUMERATION

Performance is assessed on two criteria : precision and trueness.

The assigned value of the contamination used to assess the trueness and the reference 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 characterize by its own assigned value.

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

for proficiency assessment (robust estimation of the standard deviation obtained by participants).

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

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

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



3.1.1. MICROORGANISMS AT 30°C

None significant effect of the analysis technique has been highlighted.

| Microorganisms at 30°C | | | |
|---|--------|--|--|
| Assigned value of the contamination (log CFU/g) | 4.907 | | |
| Assigned value uncertainty (log CFU/g) | 0.0064 | | |
| Standard deviation for proficiency assessment (log CFU/g) | 0.0882 | | |
| Standard deviation for precision (log CFU/g) | 0.0596 | | |
| Interlaboratory's standard deviation (log CFU/g) | 0.0840 | | |
| Reproducibility standard deviation (log CFU/g) | 0.1030 | | |

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 two groups :

| Enterobacteriaceae | Group 1 | Group 2 |
|---|---------|---------|
| Assigned value of the contamination (log CFU/g) | 2.767 | 3.223 |
| Assigned value uncertainty (log CFU/g) | 0.0269 | 0.0239 |
| Standard deviation for proficiency assessment (log CFU/g) | 0.2906 | 0.1794 |
| Standard deviation for precision (log CFU/g) | 0.1 | 036 |
| Interlaboratory's standard deviation (log CFU/g) | 0.5371 | 0.4210 |
| Reproducibility standard deviation (log CFU/g) | 0.5470 | 0.4335 |

3.1.3. TOTAL COLIFORMS

A significant "effect" of the culture media, manufacturer, preparation mode and the retained dilution has been highlighted. This effect results in a contamination's difference higher than 0.3 log CFU/g, then results have been gathered in three groups :

| Total coliforms | Group 1 | Group 2 | Group 3 |
|---|---------|---------|---------|
| Assigned value of the contamination (log CFU/g) | 2.469 | 2.750 | 3.099 |
| Assigned value uncertainty (log CFU/g) | 0.0430 | 0.0365 | 0.0497 |
| Standard deviation for proficiency assessment (log CFU/g) | 0.2644 | 0.3175 | 0.2727 |
| Standard deviation for precision (log CFU/g) | | 0.1089 | |
| Interlaboratory's standard deviation (log CFU/g) | 0.2599 | 0.3137 | 0.2683 |
| Reproducibility standard deviation (log CFU/g) | 0.2798 | 0.3304 | 0.2876 |



3.1.4. THERMOTOLERANT COLIFORMS

A significant "effect" of the diluent 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 :

| Thermotolerant coliforms | Group 1 | Group 2 |
|---|---------|---------|
| Assigned value of the contamination (log CFU/g) | 2.560 | 2.863 |
| Assigned value uncertainty (log CFU/g) | 0.0310 | 0.0672 |
| Standard deviation for proficiency assessment (log CFU/g) | 0.3047 | 0.3688 |
| Standard deviation for precision (log CFU/g) | 0.12 | 253 |
| Interlaboratory's standard deviation (log CFU/g) | 0.2995 | 0.3645 |
| Reproducibility standard deviation (log CFU/g) | 0.3169 | 0.3789 |

3.1.5. ESCHERICHIA COLI

A significant "effect" of the culture media, manufacturer and the plating method 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) | 2.228 | | |
| Assigned value uncertainty (log CFU/g) | 0.0128 | | |
| Standard deviation for proficiency assessment (log CFU/g) | 0.1710 | | |
| Standard deviation for precision (log CFU/g) | 0.1312 | | |
| Interlaboratory's standard deviation (log CFU/g) | 0.1606 | | |
| Reproducibility standard deviation (log CFU/g) | 0.2074 | | |

3.1.6. ANAEROBIC SULFITE-REDUCING BACTERIA

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

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

| Anaerobic sulfite-reducing bacteria | | |
|---|--------|--|
| Assigned value of the contamination (log CFU/g) | 2.170 | |
| Assigned value uncertainty (log CFU/g) | 0.0173 | |
| Standard deviation for proficiency assessment (log CFU/g) | 0.2044 | |
| Standard deviation for precision (log CFU/g) | 0.1314 | |
| Interlaboratory's standard deviation (log CFU/g) | 0.1898 | |
| Reproducibility standard deviation (log CFU/g) | 0.2309 | |

Comment :

- 7 laboratories detected ASR in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 1 to 2500 CFU/g.

- 7 laboratories detected ASR in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 10 to 2600 CFU/g.



3.1.7. CLOSTRIDIUM PERFRINGENS

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

None significant effect of the analysis technique has been highlighted.

| Clostridium perfringens | | |
|---|--------|--|
| Assigned value of the contamination (log CFU/g) | 2.143 | |
| Assigned value uncertainty (log CFU/g) | 0.0205 | |
| Standard deviation for proficiency assessment (log CFU/g) | 0.2229 | |
| Standard deviation for precision (log CFU/g) | 0.1178 | |
| Interlaboratory's standard deviation (log CFU/g) | 0.2122 | |
| Reproducibility standard deviation (log CFU/g) | 0.2427 | |

Comment :

- 4 laboratories detected *C. perfringens* in unit n°1 non-artificially contaminated by *C. perfringens* with a contamination level from 150 to 2000 CFU/g.

- 3 laboratories detected *C. perfringens* in unit n°2 non-artificially contaminated by *C. perfringens* with a contamination level from 110 to 2600 CFU/g.

3.1.8. COAGULASE POSITIVE STAPHYLOCOCCI

None significant effect of the analysis technique has been highlighted.

| Coagulase positive Staphylococci | | |
|---|--------|--|
| Assigned value of the contamination (log CFU/g) | 3.625 | |
| Assigned value uncertainty (log CFU/g) | 0.0107 | |
| Standard deviation for proficiency assessment (log CFU/g) | 0.1436 | |
| Standard deviation for precision (log CFU/g) | 0.0720 | |
| Interlaboratory's standard deviation (log CFU/g) | 0.1400 | |
| Reproducibility standard deviation (log CFU/g) | 0.1574 | |

3.1.9. LISTERIA MONOCYTOGENES

Only units n°4 and 5 were artificially contaminated.

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

| Listeria monocytogenes | | |
|---|--------|--|
| Assigned value of the contamination (log CFU/g) | 3.318 | |
| Assigned value uncertainty (log CFU/g) | 0.0099 | |
| Standard deviation for proficiency assessment (log CFU/g) | 0.1206 | |
| Standard deviation for precision (log CFU/g) | 0.0758 | |
| Interlaboratory's standard deviation (log CFU/g) | 0.1080 | |
| Reproducibility standard deviation (log CFU/g) | 0.1319 | |



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°3, 4 and 5 were artificially contaminated.

289 laboratories obtained correct results.

6 laboratories obtained false positive results (respectively 5 and 3 false-positive for units n°1 and 2). 15 laboratories obtained false negative results (respectively 9, 9 and 7 false-negative for units n°3, 4 and 5).

3.2.2. DETECTION - LISTERIA MONOCYTOGENES

Only units n°4 and 5 were artificially contaminated.

267 laboratories obtained correct results.

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

4 laboratories obtained false negative results (respectively 2 and 4 false-negative for units n°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 51th 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.