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PROFICIENCY TEST « RAEMA »

SCHEME N° 76 A
(5th JUNE 2023)
GENERAL REPORT

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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

157 laboratories participated to the 76Ath Gel scheme on 5th June 2023 (J0).
We received **157** answers (100%).

1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+8	J0+9
Nb of laboratories	2	99	39	9	2	2	1	2	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of 1.10^6 cfu/g ;
- one sample included a strain of *Pseudomonas sp.* at a concentration level of 2.10^4 cfu/g ;
- one sample included a strain of *Bacillus cereus* at a concentration level of 1.10^5 cfu/g ;
- one sample included a strain of *Penicillium* at a concentration level of 7.10^2 cfu/g and a strain of *Rhodotorula rubra* at a concentration level of 2.10^4 cfu/g ;

1.3.2. SIZE

Samples were composed of a gel and distributed in bottles containing 50 grammes.

1.3.3. HOMOGENEITY AND STABILITY TEST OF THE CONTAMINATION

A check of the contamination's homogeneity was realized on 10 samples per numeration in duplicate for all flora.
The contamination's stability was checked by enumeration of all flora on 8 June (J0+3), 12 June (J0+7) and 19 June 2023 (J0+14).
These checks were realized by a subcontractor accredited by Cofrac for *Bacillus cereus,* lactic bacteria and Yeast/Mould. The check of *Pseudomonas* was realized by the same subcontractor but not covered by Cofrac accreditation.
Homogeneity of samples has been validated except for Yeast/Moulds and Yeast. For these parameters, inter-samples standard deviation has been included in the calculation of standard deviation for proficiency assessment (ISO 13528 §B.2.5.a).
Stability of samples has been validated.

1.3.4 FLORA FOR ENUMERATION

Enumeration of the following flora was proposed:
- lactic acid bacteria
- *Pseudomonas*
- *Bacillus cereus*
- Yeast - Moulds analyzed together
- Yeast
- Moulds

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1.4. EXECUTION OF ANALYZES

1.4.1 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

156 laboratories (99.4%) specified it.
The average temperature is **4.3°C** with a standard deviation of 2.4°C. The minimum temperature indicated is 2.0°C and the maximum one is 24.0°C.

Remark: Please note that samples must be conserved at 4°C on receipt, before analysis. They should not be frozen.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF TEST SAMPLE

157 laboratories (100%) specified it.
The average size is **14.1 g** with a standard deviation of 6.5 g. The minimum size indicated is 1.2 g and the maximum one is 30.0 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

156 laboratories (99.4%) specified it.
153 laboratories (97.5%) prepare the initial suspension with adding diluent to gel.
3 laboratories (1.9%) prepare the initial suspension in another way.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

155 laboratories (98.7%) specified it.
144 laboratories (91.7%) use Buffered Peptone Water for the initial suspension.
9 laboratories (5.7%) use Peptone salt solution for the initial suspension.
2 laboratories (1.3%) used another diluent for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

157 laboratories (100%) specified it.
152 laboratories (96.8%) homogenize their sampling with a Stomacher[ND].
2 laboratories (1.3%) used a manual homogenization.
2 laboratories (1.3%) used a Vortex mixer.
1 laboratory (0.6%) used another technique.

The average duration is **2.5 min** with a standard deviation of 1.0 min. The data 10, 20, 30 and 35 min given by 6 laboratories were not taken into account for this calculation. The minimum duration indicated is 0.5 min and the maximum one is 6.0 min.

2.5. LACTIC ACID BACTERIA

117 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

117 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J0+10	J0+11
Nb of laboratories	21	31	17	12	1	22	9	2	2

RESUSCITATION'S CONDITIONS

21 laboratories specified a duration of 0 min (or did not specify it) for the resuscitation step, they are not taken into account for the calculation.

- DURATION

96 laboratories specified it.

The average duration is **18.7 min** with a standard deviation of 11.8 min. The minimum duration indicated is 1 min and the maximum one is 60 min. The data 120 given by 2 laboratories was not taken into account for this calculation.

- TEMPERATURE

96 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 3.2°C. The minimum temperature indicated is 4.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
ISO / NF EN ISO 15214	83
NM ISO 15214	13
TEMPO LAB	9
AFNOR 3M 01/19-11/17	8
Other	4

Culture medium	Nb laboratories
MRS pH 5.7	92
TEMPO LAB	9
Petrifilm	8
MRS pH 6.4	7
Other	1

Preparation	Nb laboratories
Home made	28
Ready to use not pre-poured	68
Ready to use, plate, film, card	21

Plating method	Nb laboratories
Surface (agar plate, film)	13
Pour	92
Culture medium for card	9

Incubation temperature	Nb laboratories
30°C	116
37°C	1

Incubation duration	Nb laboratories
69 – 72 h	97
44 – 48 h	19
88 h	1

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2.6. PSEUDOMONAS

80 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

80 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J0+9	J0+10	J0+11
Nb of laboratories	13	27	11	3	1	14	5	3	2	1

RESUSCITATION'S CONDITIONS

15 laboratories specified a duration of 0 min (or did not specify it) for the resuscitation step, they are not taken into account for the calculation.

- DURATION

65 laboratories specified it.

The average duration is **19.8 min** with a standard deviation of 13.1 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

65 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.6°C. The minimum temperature indicated is 8.0°C and the maximum one is 27.0°C.

Method	Nb laboratories
ISO / NF EN ISO 13720	49
AFNOR BKR 23/09-05/15	22
NM ISO 13720	7
Other	2

Incubation temperature	Nb laboratories
25°C	56
30°C	22
22°C	1
37°C	1

Culture medium	Nb laboratories
CFC	57
Rhapsody agar	22
Other	0

Incubation duration	Nb laboratories
44 - 48 h	79
72 h	1

Preparation	Nb laboratories
Home made	21
Ready to use not pre-poured	29
Ready to use, plate, film, card	30

Confirmation test	Nb laboratories
None	30
Oxydase	47
Other	2

2.7. BACILLUS CEREUS

127 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

126 laboratories specified it.

Analysis time	J0	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J+9	J0+10	J0+11
Nb of laboratories	1	19	35	24	5	1	27	8	3	2	1

RESUSCITATION'S CONDITIONS

22 laboratories specified a duration of 0 min (or did not specify it) for the resuscitation step, they are not taken into account for the calculation.

- DURATION

105 laboratories specified it.

The average duration is **20.1 min** with a standard deviation of 12.8 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

The data 120 given by 2 laboratories was not taken into account for this calculation.

- TEMPERATURE

105 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 2.9°C. The minimum temperature indicated is 4.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
ISO / NF EN ISO 7932/A1	49
AFNOR AES 10/10-07/10	27
AFNOR BKR 23/06-02/10	23
NM ISO 7932	13
Microval 2014LR47	6
AFNOR BRD 07/26-03/19	3
Other	6

Culture medium	Nb laboratories
Mossel	63
BACARA	28
COMPASS *Bacillus cereus* Agar	24
TEMPO BC	6
RAPID'B. cereus	3
Other	3

Preparation	Nb laboratories
Home made	24
Ready to use not pre-poured	15
Ready to use, plate, film, card	88

Plating method	Nb laboratories
Surface (agar plate, film)	107
Pour	13
Culture medium for card	6

Incubation temperature	Nb laboratories
30°C	125
37°C	2

Incubation duration	Nb laboratories
21 – 25 h	78
42 – 48 h	48
18 h	1

Confirmation test	Nb laboratories
None	67
Biochemical (including hemolysis)	57
Other	1

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2.8. YEAST / MOULDS

70 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

69 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8
Nb of laboratories	6	24	17	7	1	11	3

RESUSCITATION'S CONDITIONS

10 laboratories specified a duration of 0 min (or did not specify it) for the resuscitation step, they are not taken into account for the calculation.

- DURATION

60 laboratories specified it.

The average duration is **19.2 min** with a standard deviation of 12.2 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

60 laboratories specified it.

The average temperature is **21.6°C** with a standard deviation of 2.9°C. The minimum temperature indicated is 8.0°C and the maximum one 30.0°C.

The data 100 given by 1 laboratory was not taken into account for this calculation.

Method	Nb laboratories
NF V08-059	40
→ *NM 08.0.123* [(1)]	8
AFNOR BKR 23/11-12/18	8
ISO / NF ISO 21527-1	7
AFNOR 3M 01/13-07/14	4
NM ISO 21527-1	1
Other	2

Culture medium	Nb laboratories
YGC	35
Chloramphenicol glucose agar	11
Symphony	8
DRBC	5
Petrifilm	4
OGA	4
Other	2

[(1)] *Similar method to NF V08-059 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

Preparation	Nb laboratories
Home made	22
Ready to use not pre-poured	40
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	20
Pour	48
Culture medium for card	0

Incubation temperature	Nb laboratories
25°C	64
20 - 22°C	4
30°C	2

Incubation duration	Nb laboratories
112 - 120 h	57
69 - 72 h	12
96 h	1

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2.9. YEAST

71 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

70 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+10	J0+11
Nb of laboratories	9	15	16	10	14	4	1	1

RESUSCITATION'S CONDITIONS

12 laboratories specified a duration of 0 min (or did not specify it) for the resuscitation step, they are not taken into account for the calculation.

- DURATION

59 laboratories specified it.

The average duration is **21.5 min** with a standard deviation of 14.0 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

The data 120 given by 1 laboratory was not taken into account for this calculation.

- TEMPERATURE

59 laboratories specified it.

The average temperature is **21.7°C** with a standard deviation of 2.3°C. The minimum temperature indicated is 18.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
NF V08-059	34
→ *NM 08.0.123* [(1)]	10
AFNOR BKR 23/11-12/18	8
ISO / NF EN ISO 21527-1	8
AFNOR 3M 01/13-07/14	5
NM ISO 21527-1	2
Other	4

Culture medium	Nb laboratories
YGC	31
Chloramphenicol glucose agar	11
Symphony	9
DRBC	6
Petrifilm	5
OGA	3
Other	5

Preparation	Nb laboratories
Home made	15
Ready to use not pre-poured	47
Ready to use, plate, film, card	9

Plating method	Nb laboratories
Surface (agar plate, film)	21
Pour	46
Culture medium for card	0

Incubation temperature	Nb laboratories
25°C	66
20 - 22°C	5

Incubation duration	Nb laboratories
120 h	49
69 - 72 h	16
96 h	6

[(1)] *Similar method to NF V08-059 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

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2.10. MOULDS

71 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

70 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+10	J0+11
Nb of laboratories	9	15	16	10	14	4	1	1

RESUSCITATION'S CONDITIONS

12 laboratories specified a duration of 0 min (or did not specify it) for the resuscitation step, they are not taken into account for the calculation.

- DURATION

59 laboratories specified it.

The average duration is **21.5 min** with a standard deviation of 14.0 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

The data 120 given by 1 laboratory was not taken into account for this calculation.

- TEMPERATURE

59 laboratories specified it.

The average temperature is **21.7°C** with a standard deviation of 2.3°C. The minimum temperature indicated is 18.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
NF V08-059	34
→ *NM 08.0.123* [(1)]	10
AFNOR BKR 23/11-12/18	8
ISO / NF EN ISO 21527-1	8
AFNOR 3M 01/13-07/14	5
NM ISO 21527-1	2
Other	4

Culture medium	Nb laboratories
YGC	31
Chloramphenicol glucose agar	11
Symphony	9
DRBC	6
Petrifilm	5
OGA	3
Other	5

[(1)] *Similar method to NF V08-059 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

Preparation	Nb laboratories
Home made	15
Ready to use not pre-poured	47
Ready to use, plate, film, card	9

Plating method	Nb laboratories
Surface (agar plate, film)	21
Pour	46
Culture medium for card	0

Incubation temperature	Nb laboratories
25°C	66
20 - 22°C	5

Incubation duration	Nb laboratories
120 h	49
69 - 72 h	16
96 h	6

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3. ASSESSMENT OF PERFORMANCE (INDIVIDUEL REPORTS)

Performance is assessed on **trueness**.
The assigned value of the contamination used to assess the trueness is the consensual value obtained with the results of all the participants. This value is obtained by a robust estimation method 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 result for the contaminated unit, 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 >10 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, 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).

TRUENESS
The trueness reflects the closeness of your results to the contamination’s assigned value of samples. It has been evaluated for all enumerated flora. Your result m_i is compared to the contamination’s assigned value, X_{pt}, obtained with algorithm A from the standard ISO 13528 applied to all laboratories results included in the statistical analysis.

A z score is then calculated with the following formula : $z_i = \frac{m_i - X_{pt}}{\sigma_{pt}}$, where σ_{pt} is the standard deviation for proficiency assessment (robust estimation of the standard deviation obtained by participants). When groups are constituted, each one is characterized by its own contamination’s assigned value.
The standard ISO 13528 specifies that z score included between -2 and +2 must be considered as satisfactory signal. A z score included between -2 and -3 or between +2 and +3 must be considered as a warning signal. A z score lower than -3 or higher than +3 must be considered as an action signal

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),
- histogram for the studied parameter (results of laboratories) with an asterisk indicating the location of your result,
- when necessary, your group in relation to the technique used,
- z score,
- number of laboratories which made analysis (and belonging to your group),
- number of laboratories included in the statistical analysis,
- assigned value of the contamination and standard deviation for proficiency assessment,
- number of laboratories with a satisfactory signal,
- number of laboratories with a warning signal,
- number of laboratories with an action signal.

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3.1. LACTIC ACID BACTERIA

None significant effect of the analysis technique has been highlighted.

Lactic acid bacteria	
Number of laboratories included in the statistical analysis	115
Assigned value of the contamination (log cfu/g)	6.003
Uncertainty of assigned value (log cfu/g)	0.0295
Standard deviation for proficiency assessment (log cfu/g)	0.2534

3.2. PSEUDOMONAS

A significant “effect” of the incubation temperature 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 :

Pseudomonas	Group 1	Group 2
Number of laboratories included in the statistical analysis	57	22
Assigned value of the contamination (log cfu/g)	4.550	4.216
Uncertainty of assigned value (log cfu/g)	0.0532	0.0732
Standard deviation for proficiency assessment (log cfu/g)	0.3216	0.2745

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

Bacillus cereus	
Number of laboratories included in the statistical analysis	125
Assigned value of the contamination (log cfu/g)	5.113
Uncertainty of assigned value (log cfu/g)	0.0254
Standard deviation for proficiency assessment (log cfu/g)	0.2271

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Number of laboratories included in the statistical analysis	69
Assigned value of the contamination (log cfu/g)	4.346
Uncertainty of assigned value (log cfu/g)	0.0406
Standard deviation for proficiency assessment (log cfu/g)	0.2697

Comment : We specify that the homogeneity criterion is unsatisfactory for Yeast/Moulds enumeration. Inter-samples standard deviation has then been included in the calculation of standard deviation for proficiency assessment (ISO 13528 §B.2.5.a).

3.5. YEAST

None significant effect of the analysis technique has been highlighted.

Yeast	
Number of laboratories included in the statistical analysis	69
Assigned value of the contamination (log cfu/g)	4.289
Uncertainty of assigned value (log cfu/g)	0.0477
Standard deviation for proficiency assessment (log cfu/g)	0.3170

Comment : We specify that the homogeneity criterion is unsatisfactory for Yeast enumeration. Inter-samples standard deviation has then been included in the calculation of standard deviation for proficiency assessment (ISO 13528 §B.2.5.a).

3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

Moulds	
Number of laboratories included in the statistical analysis	69
Assigned value of the contamination (log cfu/g)	2.898
Uncertainty of assigned value (log cfu/g)	0.0249
Standard deviation for proficiency assessment (log cfu/g)	0.1658

3.7. EVOLUTION OF PERFORMANCE

You will find, at the end of the individual report, graphs representing evolution of your performance on different tests since the 61A 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 either positive or negative.

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