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PROFICIENCY TEST « RAEMA »

SCHEME N° 73 A
(6th DECEMBER 2021)
GENERAL REPORT

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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

142 laboratories participated to the 73Ath Gel scheme on 6th December 2021 (J0).
We received **140** answers.

1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+8	J0+9	J0+10
Nb of laboratories	1	100	23	6	1	4	1	2

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of 1.10^5 cfu/g ;
- one sample included a strain of *Pseudomonas sp.* at a concentration level of 2.10^3 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 2.10^3 cfu/g and a strain of *Rhodotorula rubra* at a concentration level of 1.10^4 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 9 December (J0+3), 13 December (J0+7) and 20 December 2021 (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.
Stability of samples has been validated. Homogeneity of samples has been validated except for Yeast / Moulds and Yeast. For these two parameters, inter-samples standard deviation has been included in the calculation of standard deviation for proficiency assessment (ISO 13528 §B.2.5.a).

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

139 laboratories specified it.
The average temperature is **4.0°C** with a standard deviation of 1.8°C. The minimum temperature indicated is 2.0°C and the maximum one is 20.8°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

139 laboratories specified it.
The average size is **13.8 g** with a standard deviation of 6.3 g. The minimum size indicated is 1 g and the maximum one is 25 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

139 laboratories specified it.
137 laboratories prepare the initial suspension with adding diluent to gel.
2 laboratories prepare the initial suspension in another way.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

139 laboratories specified it.
121 laboratories use Buffered Peptone Water for the initial suspension.
7 laboratories use Peptone salt solution for the initial suspension.
11 laboratories used another diluent for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

140 laboratories specified it.
132 laboratories homogenize their sampling with a StomacherND.
8 laboratories used another technique.

The average duration is **2.3 min** with a standard deviation of 1.0 min. The data 10, 15, 20, 30, 40 and 60 min given by 8 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

109 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

107 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10	J0+18
Nb of laboratories	18	29	17	9	20	8	4	1	1

RESUSCITATION'S CONDITIONS

19 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

90 laboratories specified it.

The average duration is **20.3 min** with a standard deviation of 12.5 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

90 laboratories specified it.

The average temperature is **21.0°C** with a standard deviation of 3.1°C. The minimum temperature indicated is 4°C and the maximum one is 30°C.

Method	Nb laboratories
ISO / NF EN ISO 15214	76
NM ISO 15214	11
AFNOR 3M 01/19-11/17	9
TEMPO LAB	8
Other	5

Culture medium	Nb laboratories
MRS pH 5.7	89
Petrifilm	9
TEMPO LAB	9
Other	1

Preparation	Nb laboratories
Home made	26
Ready to use not pre-poured	62
Ready to use, plate, film, card	21

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	82
Culture medium for card	9

Incubation temperature	Nb laboratories
30°C	107
37°C	2

Incubation duration	Nb laboratories
69 – 75 h	87
40 – 48 h	21
24 h	1

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2.6. PSEUDOMONAS

73 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

73 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	13	23	13	4	12	6	1	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

58 laboratories specified it.

The average duration is **18.4 min** with a standard deviation of 11.8 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

58 laboratories specified it.

The average temperature is **21.0°C** with a standard deviation of 2.4°C. The minimum temperature indicated is 7.8°C and the maximum one is 25°C.

Method	Nb laboratories
ISO / NF EN ISO 13720	46
AFNOR BKR 23/09-05/15	17
NM ISO 13720	6
Other	4

Culture medium	Nb laboratories
CFC	55
Rhapsody agar	17
Other	1

Preparation	Nb laboratories
Home made	19
Ready to use not pre-poured	31
Ready to use, plate, film, card	23

Incubation temperature	Nb laboratories
25°C	55
30°C	17
22°C	1

Incubation duration	Nb laboratories
44 - 48 h	71
40 - 41 h	2

Confirmation test	Nb laboratories
None	29
Oxydase	43
Other	0

2.7. BACILLUS CEREUS

115 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

113 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	18	30	23	5	23	8	5	1

RESUSCITATION'S CONDITIONS

20 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

95 laboratories specified it.

The average duration is **21.8 min** with a standard deviation of 13.8 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

96 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 2.9°C. The minimum temperature indicated is 4°C and the maximum one is 30°C.

Method	Nb laboratories
ISO / NF EN ISO 7932/A1	53
AFNOR BKR 23/06-02/10	21
AFNOR AES 10/10-07/10	19
NM ISO 7932	12
Microval 2014LR47	6
Other	4

Culture medium	Nb laboratories
Mossel	64
COMPASS *Bacillus cereus* Agar	23
BACARA	19
TEMPO BC	6
Other	3

Preparation	Nb laboratories
Home made	19
Ready to use not pre-poured	13
Ready to use, plate, film, card	83

Plating method	Nb laboratories
Surface (agar plate, film)	99
Pour	8
Culture medium for card	6

Incubation temperature	Nb laboratories
30°C	113
37°C	2

Incubation duration	Nb laboratories
20 - 24 h	66
42 – 48 h	46
18 – 19 h	2
2 h	1

Confirmation test	Nb laboratories
None	59
Biochemical (including hemolysis)	54
Other	0

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2.8. YEAST / MOULDS

64 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

64 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10	J0+11
Nb of laboratories	11	14	15	4	10	5	1	3	1

RESUSCITATION'S CONDITIONS

13 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

51 laboratories specified it.

The average duration is **17.4 min** with a standard deviation of 11.3 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

51 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 3.2°C. The minimum temperature indicated is 7.8°C and the maximum one 30°C. The data 100 min given by 1 laboratory was not taken into account for this calculation.

Method	Nb laboratories
NF V08-059	37
→ *NM 08.0.123* [(1)]	7
AFNOR BKR 23/11-12/18	10
AFNOR 3M 01/13-07/14	4
ISO / NF ISO 21527-1	2
Other	3

Culture medium	Nb laboratories
YGC	31
Symphony	10
Chloramphenicol glucose agar	9
OGA	7
Petrifilm	4
DRBC	1
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	20
Ready to use not pre-poured	34
Ready to use, plate, film, card	10

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	45
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	60
30°C	3
20°C	1

Incubation duration	Nb laboratories
113 - 120 h	44
69 - 72 h	15
96 h	4
60 h	1

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2.9. YEAST

54 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

54 laboratories specified it.

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

RESUSCITATION'S CONDITIONS

9 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

45 laboratories specified it.

The average duration is **22.1 min** with a standard deviation of 14.0 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

45 laboratories specified it.

The average temperature is **21.6°C** with a standard deviation of 4.2°C. The minimum temperature indicated is 18°C and the maximum one is 47°C.

Method	Nb laboratories
NF V08-059	28
→ *NM 08.0.123* [(1)]	7
AFNOR BKR 23/11-12/18	6
AFNOR 3M 01/13-07/14	4
ISO / NF EN ISO 21527-1	3
NM ISO 21527-1	2
Other	4

Culture medium	Nb laboratories
YGC	23
Chloramphenicol glucose agar	9
Symphony	7
OGA	4
Petrifilm	4
DRBC	4
Other	3

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

Preparation	Nb laboratories
Home made	12
Ready to use not pre-poured	35
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	17
Pour	37
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	52
20 - 22°C	2

Incubation duration	Nb laboratories
120 h	34
69 - 72 h	14
96 h	5
161 h	1

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2.10. MOULDS

54 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

54 laboratories specified it.

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

RESUSCITATION'S CONDITIONS

9 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

45 laboratories specified it.

The average duration is **22.1 min** with a standard deviation of 14.0 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

45 laboratories specified it.

The average temperature is **21.6°C** with a standard deviation of 4.2°C. The minimum temperature indicated is 18°C and the maximum one is 47°C.

Method	Nb laboratories
NF V08-059	28
→ *NM 08.0.123* [(1)]	7
AFNOR BKR 23/11-12/18	6
AFNOR 3M 01/13-07/14	4
ISO / NF EN ISO 21527-1	3
NM ISO 21527-1	2
Other	4

Culture medium	Nb laboratories
YGC	23
Chloramphenicol glucose agar	9
Symphony	7
OGA	4
Petrifilm	4
DRBC	4
Other	3

Preparation	Nb laboratories
Home made	12
Ready to use not pre-poured	35
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	17
Pour	37
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	52
20 - 22°C	2

Incubation duration	Nb laboratories
120 h	34
69 - 72 h	14
96 h	5
161 h	1

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

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.

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	103
Assigned value of the contamination (log cfu/g)	4.996
Uncertainty of assigned value (log cfu/g)	0.0290
Standard deviation for proficiency assessment (log cfu/g)	0.2355

3.2. PSEUDOMONAS

A significant “effect” of the confirmation test has been highlighted. This effect results in a contamination’s difference higher than 0.3 log cfu/g, then results have been separated in two groups :

Pseudomonas	Group 1	Group 2
Number of laboratories included in the statistical analysis	27	43
Assigned value of the contamination (log cfu/g)	3.216	3.531
Uncertainty of assigned value (log cfu/g)	0.0823	0.0530
Standard deviation for proficiency assessment (log cfu/g)	0.3420	0.2780

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

Bacillus cereus	
Number of laboratories included in the statistical analysis	109
Assigned value of the contamination (log cfu/g)	5.026
Uncertainty of assigned value (log cfu/g)	0.0343
Standard deviation for proficiency assessment (log cfu/g)	0.2865

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Number of laboratories included in the statistical analysis	57
Assigned value of the contamination (log cfu/g)	4.078
Uncertainty of assigned value (log cfu/g)	0.0405
Standard deviation for proficiency assessment (log cfu/g)	0.2446

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	52
Assigned value of the contamination (log cfu/g)	3.962
Uncertainty of assigned value (log cfu/g)	0.0656
Standard deviation for proficiency assessment (log cfu/g)	0.3787

Comment :
- We specify that the homogeneity criterion is unsatisfactory for Yeast enumeration. Inter-samples standard deviation has then be 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	52
Assigned value of the contamination (log cfu/g)	3.272
Uncertainty of assigned value (log cfu/g)	0.0364
Standard deviation for proficiency assessment (log cfu/g)	0.2101

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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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