

PROFICIENCY TEST « RAEMA »



SCHEME N° 74 A (9th MAY 2022) GENERAL REPORT

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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

145 laboratories participated to the 74Ath Gel scheme on 9th May 2022 (J0).

We received 144 answers.

1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+8	J0+10
Nb of laboratories	5	109	22	6	1	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of 2.10^4 cfu/g ;
- one sample included a strain of *Pseudomonas* sp. at a concentration level of 3.10^3 cfu/g ;
- one sample included a strain of *Bacillus cereus* at a concentration level of 6.10^4 cfu/g ;
- one sample included a strain of *Penicillium* at a concentration level of 1.10^3 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 12 May (J0+3), 16 May (J0+7) and 23 May 2022 (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 *Bacillus cereus*. For this parameter, 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 except for lactic acid bacteria. In accordance with the MET50_P1g procedure, the impact has been assessed; there is no impact on participants' results.

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

1.4. EXECUTION OF ANALYZES

1.4.1 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

144 laboratories specified it.

The average temperature is **4.3°C** with a standard deviation of 3.1°C. The minimum temperature indicated is 2.0°C and the maximum one is 26.0°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

144 laboratories specified it.

The average size is **13.7 g** with a standard deviation of 6.1 g. The minimum size indicated is 4 g and the maximum one is 25 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

143 laboratories specified it.

142 laboratories prepare the initial suspension with adding diluent to gel.

1 laboratory prepare the initial suspension in another way.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

143 laboratories specified it.

132 laboratories use Buffered Peptone Water for the initial suspension.

9 laboratories use Peptone salt solution for the initial suspension.

2 laboratories used another diluent for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

143 laboratories specified it.

139 laboratories homogenize their sampling with a StomacherND.

4 laboratories used another technique.

The average duration is **2.3 min** with a standard deviation of 1.1 min. The data 20, 30 and 60 min given by 3 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

111 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

111 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J0+9	J0+15
Nb of laboratories	24	31	14	7	1	20	9	4	1

RESUSCITATION'S CONDITIONS

18 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

93 laboratories specified it.

The average duration is **17.9 min** with a standard deviation of 8.6 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

93 laboratories specified it.

The average temperature is **21.1°C** with a standard deviation of 2.6°C. The minimum temperature indicated is 5.8°C and the maximum one is 30°C.

Method	Nb laboratories
ISO / NF EN ISO 15214	82
NM ISO 15214	10
AFNOR 3M 01/19-11/17	8
TEMPO LAB	7
Other	4

Culture medium	Nb laboratories
MRS pH 5.7	94
Petrifilm	8
TEMPO LAB	7
Other	2

Preparation	Nb laboratories
Home made	24
Ready to use not pre-poured	71
Ready to use, plate, film, card	16

Plating method	Nb laboratories
Surface (agar plate, film)	17
Pour	85
Culture medium for card	7

Incubation temperature	Nb laboratories
30°C	110
37°C	1

Incubation duration	Nb laboratories
70 – 72 h	93
44 – 48 h	18

2.6. PSEUDOMONAS

77 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

77 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+11	J0+15
Nb of laboratories	20	23	7	5	12	5	3	1	1

RESUSCITATION'S CONDITIONS

11 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

66 laboratories specified it.

The average duration is **16.7 min** with a standard deviation of 10.8 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

66 laboratories specified it.

The average temperature is **20.9°C** with a standard deviation of 2.4°C. The minimum temperature indicated is 5.8°C and the maximum one is 25.0°C.

Method	Nb laboratories
ISO / NF EN ISO 13720	50
AFNOR BKR 23/09-05/15	19
NM ISO 13720	6
Other	2

Culture medium	Nb laboratories
CFC	58
Rhapsody agar	19
Other	0

Preparation	Nb laboratories
Home made	19
Ready to use not pre-poured	32
Ready to use, plate, film, card	26

Incubation temperature	Nb laboratories
25°C	56
30°C	20
22°C	1

Incubation duration	Nb laboratories
44 - 48 h	75
72 h	2

Confirmation test	Nb laboratories
None	29
Oxydase	46
Other	0

2.7. BACILLUS CEREUS

112 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

112 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J0+9
Nb of laboratories	27	34	16	4	1	22	6	2

RESUSCITATION'S CONDITIONS

16 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 **19.8 min** with a standard deviation of 13.2 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

96 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 2.2°C. The minimum temperature indicated is 18°C and the maximum one is 30°C.

Method	Nb laboratories
ISO / NF EN ISO 7932/A1	45
AFNOR AES 10/10-07/10	26
AFNOR BKR 23/06-02/10	22
NM ISO 7932	9
Microval 2014LR47	4
Other	6

Culture medium	Nb laboratories
Mossel	57
BACARA	26
COMPASS <i>Bacillus cereus</i> Agar	20
TEMPO BC	4
Other	5

Preparation	Nb laboratories
Home made	17
Ready to use not pre-poured	17
Ready to use, plate, film, card	78

Plating method	Nb laboratories
Surface (agar plate, film)	97
Pour	12
Culture medium for card	2

Incubation temperature	Nb laboratories
30°C	110
37°C	2

Incubation duration	Nb laboratories
21 - 24 h	67
40 – 48 h	43
18 h	2

Confirmation test	Nb laboratories
None	59
Biochemical (including hemolysis)	51
Other	0

2.8. YEAST / MOULDS

58 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

58 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9
Nb of laboratories	13	14	16	4	6	3	2

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

48 laboratories specified it.

The average duration is **17.2 min** with a standard deviation of 10.4 min. The minimum duration indicated is 1 min and the maximum one is 45 min.

- TEMPERATURE

48 laboratories specified it.

The average temperature is **21.1°C** with a standard deviation of 3.1°C. The minimum temperature indicated is 5.8°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	35
→ NM 08.0.123 ⁽¹⁾	4
AFNOR BKR 23/11-12/18	7
AFNOR 3M 01/13-07/14	4
ISO / NF ISO 21527-1	4
NM ISO 21527-1	1
Other	3

Culture medium	Nb laboratories
YGC	31
Symphony	7
Chloramphenicol glucose agar	6
OGA	6
Petrifilm	4
DRBC	2
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	18
Ready to use not pre-poured	32
Ready to use, plate, film, card	8
Plating method	Nb laboratories
Surface (agar plate, film)	17
Pour	40
Culture medium for card	0
Incubation temperature	Nb laboratories
24 - 25°C	55
20 - 22°C	2
30°C	1
Incubation duration	Nb laboratories
117 - 128 h	46
72 h	11
96 h	1

2.9. YEAST

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+6	J0+7	J0+8	J0+9	J0+15
Nb of laboratories	13	15	10	7	1	9	4	4	1

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

54 laboratories specified it.

The average duration is **20.5 min** with a standard deviation of 14.5 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

54 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 2.1°C. The minimum temperature indicated is 18°C and the maximum one is 30°C.

Method	Nb laboratories
NF V08-059	30
→ NM 08.0.123 ⁽¹⁾	7
AFNOR BKR 23/11-12/18	9
ISO / NF EN ISO 21527-1	7
AFNOR 3M 01/13-07/14	5
NM ISO 21527-1	1
Other	5

Culture medium	Nb laboratories
YGC	29
Symphony	10
Chloramphenicol glucose agar	8
DRBC	6
Petrifilm	5
OGA	4
Other	2

Preparation	Nb laboratories
Home made	11
Ready to use not pre-poured	43
Ready to use, plate, film, card	10
Plating method	Nb laboratories
Surface (agar plate, film)	19
Pour	43
Culture medium for card	0
Incubation temperature	Nb laboratories
24 - 25°C	61
20 - 22°C	3
Incubation duration	Nb laboratories
120 - 125 h	45
71 - 72 h	17
96 h	2

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

2.10. 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+6	J0+7	J0+8	J0+9	J0+15
Nb of laboratories	13	15	10	7	1	9	4	4	1

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

54 laboratories specified it.

The average duration is **20.5 min** with a standard deviation of 14.5 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

54 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 2.1°C. The minimum temperature indicated is 18°C and the maximum one is 30°C.

Method	Nb laboratories
NF V08-059	30
→ NM 08.0.123 ⁽¹⁾	7
AFNOR BKR 23/11-12/18	9
ISO / NF EN ISO 21527-1	7
AFNOR 3M 01/13-07/14	5
NM ISO 21527-1	1
Other	5

Culture medium	Nb laboratories
YGC	29
Symphony	10
Chloramphenicol glucose agar	8
DRBC	6
Petrifilm	5
OGA	4
Other	2

Preparation	Nb laboratories
Home made	11
Ready to use not pre-poured	43
Ready to use, plate, film, card	10
Plating method	Nb laboratories
Surface (agar plate, film)	19
Pour	43
Culture medium for card	0
Incubation temperature	Nb laboratories
24 - 25°C	61
20 - 22°C	3
Incubation duration	Nb laboratories
120 - 125 h	45
71 - 72 h	17
96 h	2

⁽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	110
Assigned value of the contamination (log cfu/g)	4.295
Uncertainty of assigned value (log cfu/g)	0.0382
Standard deviation for proficiency assessment (log cfu/g)	0.3204

3.2. PSEUDOMONAS

A significant “effect” of the resuscitation’s duration 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	61	14
Assigned value of the contamination (log cfu/g)	3.519	3.203
Uncertainty of assigned value (log cfu/g)	0.0576	0.1085
Standard deviation for proficiency assessment (log cfu/g)	0.3596	0.3247

Comment : Given the number of laboratories in group 2, the uncertainty of assigned value is not negligible (ISO 13528 §9.2.1). All laboratories from group 2 obtain a satisfactory signal for z score, so there is no consequence.

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

Bacillus cereus	
Number of laboratories included in the statistical analysis	112
Assigned value of the contamination (log cfu/g)	4.793
Uncertainty of assigned value (log cfu/g)	0.0271
Standard deviation for proficiency assessment (log cfu/g)	0.2467

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

3.4. 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)	4.323
Uncertainty of assigned value (log cfu/g)	0.0491
Standard deviation for proficiency assessment (log cfu/g)	0.2964

3.5. YEAST

None significant effect of the analysis technique has been highlighted.

Yeast	
Number of laboratories included in the statistical analysis	62
Assigned value of the contamination (log cfu/g)	4.359
Uncertainty of assigned value (log cfu/g)	0.0559
Standard deviation for proficiency assessment (log cfu/g)	0.3524

3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

Moulds	
Number of laboratories included in the statistical analysis	62
Assigned value of the contamination (log cfu/g)	2.970
Uncertainty of assigned value (log cfu/g)	0.0273
Standard deviation for proficiency assessment (log cfu/g)	0.1717

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.