

PROFICIENCY TEST « RAEMA »



SCHEME N° 72 A (31th MAY 2021) GENERAL REPORT

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Comment :

This edition of 21 september 2021 cancels and replaces the edition of 26 july 2021, on account of an error in the calculation of the value of the standard deviation for proficiency assessment for parameters Yeast/Moulds and Yeast (adjustment following the unsatisfactory homogeneity criterion).

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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

143 laboratories participated to the 72Ath Gel scheme on 31th May 2021 (J0).
We received **141** answers.

1.2. DELIVERY TIME OF THE PARCEL

3 laboratories did not specify the delivery time of the parcel.

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+8	J0+11	J0+14
Nb of laboratories	1	100	21	8	3	2	1	2

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of 1.10^6 cfu/g ;
- one sample included a strain of *Pseudomonas sp.* at a concentration level of 1.10^4 cfu/g ;
- one sample included a strain of *Bacillus cereus* at a concentration level of 2.10^5 cfu/g ;
- one sample included a strain of *Penicillium* at a concentration level of 7.10^3 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 3 June (J0+3), 7 June (J0+7) and 14 June 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).

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

141 laboratories specified it.

The average temperature is **4.0°C** with a standard deviation of 1.9°C. The minimum temperature indicated is 2.0°C and the maximum one is 20.0°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

141 laboratories specified it.

The average size is **13.9 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

140 laboratories specified it.

138 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

140 laboratories specified it.

129 laboratories use Buffered Peptone Water for the initial suspension.

7 laboratories use Peptone salt solution for the initial suspension.

4 laboratories used another diluent for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

141 laboratories specified it.

136 laboratories homogenize their sampling with a StomacherND.

5 laboratories used another technique.

The average duration is **2.4 min** with a standard deviation of 1.0 min. The data 10, 20, 40 and 60 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 5.0 min.

2.5. LACTIC ACID BACTERIA

103 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

103 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	21	25	16	8	15	9	3	4	2

RESUSCITATION'S CONDITIONS

17 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

86 laboratories specified it.

The average duration is **18.5 min** with a standard deviation of 12.2 min. The minimum duration indicated is 1 min and the maximum one is 60 min. The data 120 min given by 1 laboratory was not taken into account for this calculation.

- TEMPERATURE

86 laboratories specified it.

The average temperature is **21.0°C** with a standard deviation of 3.3°C. The minimum temperature indicated is 4°C and the maximum one is 30°C.

Method	Nb laboratories
NF EN ISO 15214	73
NM ISO 15214	8
TEMPO LAB	7
AFNOR 3M 01/19-11/17	7
Other	8

Culture medium	Nb laboratories
MRS pH 5.7	87
Petrifilm	7
TEMPO LAB	7
Other	2

Preparation	Nb laboratories
Home made	23
Ready to use not pre-poured	64
Ready to use, plate, film, card	16

Plating method	Nb laboratories
Surface (agar plate, film)	14
Pour	81
Culture medium for card	7

Incubation temperature	Nb laboratories
30°C	102
37°C	1

Incubation duration	Nb laboratories
70 – 72 h	82
44 – 48 h	19
24 h	2

2.6. PSEUDOMONAS

72 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

72 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10	J0+11	J0+15
Nb of laboratories	15	21	15	2	8	6	1	1	1	2

RESUSCITATION'S CONDITIONS

14 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 **18.9 min** with a standard deviation of 12.1 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 2.7°C. The minimum temperature indicated is 5.0°C and the maximum one is 26.1°C.

Method	Nb laboratories
NF EN ISO 13720	45
AFNOR BKR 23/09-05/15	18
NM ISO 13720	5
Other	4

Culture medium	Nb laboratories
CFC	53
Rhapsody agar	19
Other	0

Preparation	Nb laboratories
Home made	16
Ready to use not pre-poured	27
Ready to use, plate, film, card	28

Incubation temperature	Nb laboratories
25°C	52
30°C	19

Incubation duration	Nb laboratories
44 - 48 h	69
42 h	1
23 h	1

Confirmation test	Nb laboratories
None	29
Oxydase	40
Other	1

2.7. BACILLUS CEREUS

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+5	J0+7	J0+8	J0+9	J0+11	J0+15
Nb of laboratories	22	27	22	5	1	19	7	4	2	2

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

91 laboratories specified it.

The average duration is **20.0 min** with a standard deviation of 12.6 min. The minimum duration indicated is 1 min and the maximum one is 60 min. The data 120 min given by 1 laboratory was not taken into account for this calculation.

- TEMPERATURE

91 laboratories specified it.

The average temperature is **21.3°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
NF EN ISO 7932/A1	53
AFNOR AES 10/10-07/10	22
AFNOR BKR 23/06-02/10	20
NM ISO 7932	9
Microval 2014LR47	3
Other	3

Culture medium	Nb laboratories
Mossel	62
BACARA	23
COMPASS <i>Bacillus cereus</i> Agar	20
TEMPO BC	3
Other	3

Preparation	Nb laboratories
Home made	20
Ready to use not pre-poured	11
Ready to use, plate, film, card	80

Plating method	Nb laboratories
Surface (agar plate, film)	101
Pour	6
Culture medium for card	3

Incubation temperature	Nb laboratories
30°C	109
37°C	2

Incubation duration	Nb laboratories
20 - 25 h	69
45 - 48 h	42

Confirmation test	Nb laboratories
None	54
Biochemical (including hemolysis)	52
Other	2

2.8. YEAST / MOULDS

63 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

63 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	10	19	10	7	6	6	3	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

51 laboratories specified it.

The average duration is **18.0 min** with a standard deviation of 12.0 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

51 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 3.7°C. The minimum temperature indicated is 5°C and the maximum one 32°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⁾	6
AFNOR BKR 23/11-12/18	8
AFNOR 3M 01/13-07/14	5
NF ISO 21527-1	4
AOAC RI 041001	2
Other	3

Culture medium	Nb laboratories
YGC	28
Symphony	9
Chloramphenicol glucose agar	8
OGA	7
Petrifilm	5
TEMPO YM	2
DRBC	1
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	18
Ready to use not pre-poured	35
Ready to use, plate, film, card	10

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	42
Culture medium for card	2

Incubation temperature	Nb laboratories
24 - 25°C	59
30°C	3
20°C	1

Incubation duration	Nb laboratories
115 - 120 h	44
69 - 72 h	16
96 h	3

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+5	J0+7	J0+8	J0+11	J0+15
Nb of laboratories	6	12	15	5	1	11	2	1	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

44 laboratories specified it.

The average duration is **20.8 min** with a standard deviation of 13.2 min. The minimum duration indicated is 1 min and the maximum one is 60 min. The data 120 min given by 1 laboratory was not taken into account for this calculation.

- TEMPERATURE

44 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 1.9°C. The minimum temperature indicated is 20°C and the maximum one is 26.1°C.

Method	Nb laboratories
NF V08-059	26
→ NM 08.0.123 ⁽¹⁾	8
AFNOR BKR 23/11-12/18	7
NF EN ISO 21527-1	4
AFNOR 3M 01/13-07/14	4
NM ISO 21527-1	1
Other	4

Culture medium	Nb laboratories
YGC	22
Symphony	9
Chloramphenicol glucose agar	8
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	11
Ready to use not pre-poured	33
Ready to use, plate, film, card	9

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	33
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	51
20°C	2

Incubation duration	Nb laboratories
120 h	36
70 - 72 h	14
96 h	2
166 h	1

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+5	J0+7	J0+8	J0+11	J0+15
Nb of laboratories	6	12	15	5	1	11	2	1	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

44 laboratories specified it.

The average duration is **20.8 min** with a standard deviation of 13.2 min. The minimum duration indicated is 1 min and the maximum one is 60 min. The data 120 min given by 1 laboratory was not taken into account for this calculation.

- TEMPERATURE

44 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 1.9°C. The minimum temperature indicated is 20°C and the maximum one is 26.1°C.

Method	Nb laboratories
NF V08-059	26
→ NM 08.0.123 ⁽¹⁾	8
AFNOR BKR 23/11-12/18	7
NF EN ISO 21527-1	4
AFNOR 3M 01/13-07/14	4
NM ISO 21527-1	1
Other	4

Culture medium	Nb laboratories
YGC	22
Symphony	9
Chloramphenicol glucose agar	8
OGA	4
Petrifilm	4
DRBC	4
Other	3

Preparation	Nb laboratories
Home made	11
Ready to use not pre-poured	33
Ready to use, plate, film, card	9

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	33
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	51
20°C	2

Incubation duration	Nb laboratories
120 h	36
70 - 72 h	14
96 h	2
166 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 be 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	96
Assigned value of the contamination (log CFU/g)	6.265
Uncertainty of assigned value (log CFU/g)	0.0655
Standard deviation for proficiency assessment (log CFU/g)	0.5135

3.2. PSEUDOMONAS

A significant "effect" of the confirmation test 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:

<i>Pseudomonas</i>	
Number of laboratories included in the statistical analysis	69
Assigned value of the contamination (log CFU/g)	3.913
Uncertainty of assigned value (log CFU/g)	0.0550
Standard deviation for proficiency assessment (log CFU/g)	0.3655

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

<i>Bacillus cereus</i>	
Number of laboratories included in the statistical analysis	106
Assigned value of the contamination (log CFU/g)	5.391
Uncertainty of assigned value (log CFU/g)	0.0341
Standard deviation for proficiency assessment (log CFU/g)	0.2806

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Number of laboratories included in the statistical analysis	59
Assigned value of the contamination (log CFU/g)	4.434
Uncertainty of assigned value (log CFU/g)	0.0710
Standard deviation for proficiency assessment (log CFU/g)	0.4362

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

3.5. YEAST

A significant “effect” of the homogeneization 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:

Yeast	Group 1	Group 2
Number of laboratories included in the statistical analysis	17	35
Assigned value of the contamination (log CFU/g)	3.915	4.421
Uncertainty of assigned value (log CFU/g)	0.1517	0.1457
Standard deviation for proficiency assessment (log CFU/g)	0.5005	0.6897

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).
- The number of laboratories included in group 1(17) does not allow to get an insignificant uncertainty of assigned value. In this group, the 3 laboratories having obtained a warning signal will be informed in their individual report.

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)	3.847
Uncertainty of assigned value (log CFU/g)	0.0384
Standard deviation for proficiency assessment (log CFU/g)	0.2215

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 regularly increasing or decreasing.