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PROFICIENCY TEST « RAEMA »

SCHEME N° 78 A
(3rd JUNE 2024)
GENERAL REPORT

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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

154 laboratories participated to the 78Ath Gel scheme on 3rd June 2024 (J0).
We received **154** answers (100%).

1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+11
Nb of laboratories	4	119	19	5	3	1	1	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of 1.10^5 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 5.10^3 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 7.10^3 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 6 June (J0+3), 10 June (J0+7) and 17 June 2024 (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 and stability of samples have 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

1.4. EXECUTION OF ANALYZES

1.4.1 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

153 laboratories (99.4%) specified it.
The average temperature is **4.2°C** with a standard deviation of 1.9°C. The minimum temperature indicated is 2.0°C and the maximum one is 20.3°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

153 laboratories (99.4%) specified it.
The average size is **14.2 g** with a standard deviation of 6.4 g. The data 1.084 g given by 1 laboratory was not taken into account for this calculation The minimum size indicated is 10 g and the maximum one is 30 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

153 laboratories (99.4%) specified it.
149 laboratories (96.8%) prepare the initial suspension with adding diluent to gel.
4 laboratories (2.6%) prepare the initial suspension in another way.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

153 laboratories (99.4%) specified it.
147 laboratories (95.5%) use Buffered Peptone Water for the initial suspension.
6 laboratories (3.9%) use Peptone salt solution for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

153 laboratories (99.4%) specified it.
148 laboratories (96.1%) homogenize their sampling with a StomacherND.
2 laboratories (1.3%) used a manual homogenization.
3 laboratories (2.0%) used a Vortex mixer.

The average duration is **2.4 min** with a standard deviation of 1.0 min. The data 10, 15, 20 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.

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2.5. LACTIC ACID BACTERIA

116 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

116 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+13
Nb of laboratories	29	27	18	7	20	13	1	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

98 laboratories specified it.

The average duration is **19.3 min** with a standard deviation of 12.8 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

98 laboratories specified it.

The average temperature is **21.0°C** with a standard deviation of 3.1°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	84
TEMPO LAB	10
NM ISO 15214	9
AFNOR 3M 01/19-11/17	9
Other	4

Culture medium	Nb laboratories
MRS pH 5.7	85
TEMPO LAB	11
MRS pH 6.4	9
Petrifilm	9
Other	1

Preparation	Nb laboratories
Home made	24
Ready to use not pre-poured	70
Ready to use, plate, film, card	22

Plating method	Nb laboratories
Surface (agar plate, film)	16
Pour	86
Transfer Tempo filler ®	9

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

Incubation duration	Nb laboratories
70 - 72 h	94
42 - 48 h	21

2.6. PSEUDOMONAS

77 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

76 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9
Nb of laboratories	19	24	16	3	6	6	3

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

63 laboratories specified it.

The average duration is **17.8 min** with a standard deviation of 11.0 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

63 laboratories specified it.

The average temperature is **21.2°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	46
AFNOR BKR 23/09-05/15	24
NM ISO 13720	5
Other	2

Culture medium	Nb laboratories
CFC	52
Rhapsody agar	24
Other	1

Preparation	Nb laboratories
Home made	18
Ready to use not pre-poured	28
Ready to use, plate, film, card	31

Incubation temperature	Nb laboratories
25°C	51
30°C	24
37°C	2

Incubation duration	Nb laboratories
43 - 48 h	73
18 - 24 h	3
72 h	1

Confirmation test	Nb laboratories
None	33
Oxydase	43

2.7. BACILLUS CEREUS

128 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

128 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	26	36	22	4	24	8	7	1

RESUSCITATION'S CONDITIONS

23 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 **20.2 min** with a standard deviation of 13.0 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

105 laboratories specified it.

The average temperature is **21.7°C** with a standard deviation of 3.9°C. The minimum temperature indicated is 4.0°C and the maximum one is 47.0°C.

Method	Nb laboratories
ISO / NF EN ISO 7932/A1	52
AFNOR BKR 23/06-02/10	28
AFNOR AES 10/10-07/10	22
NM ISO 7932/A1	8
Microval 2014LR47	7
AFNOR BRD 07/26-03/19	5
Other	6

Culture medium	Nb laboratories
Mossel	61
COMPASS *Bacillus cereus* Agar	29
BACARA	26
TEMPO BC	7
RAPID'B. cereus	5

Preparation	Nb laboratories
Home made	21
Ready to use not pre-poured	14
Ready to use, plate, film, card	93

Plating method	Nb laboratories
Surface (agar plate, film)	106
Pour	12
Transfer Tempo filler ®	6

Incubation temperature	Nb laboratories
30°C	128

Incubation duration	Nb laboratories
22 - 25 h	81
42 - 48 h	43
18 - 21 h	4

Confirmation test	Nb laboratories
None	71
Biochemical (including hemolysis)	55

2.8. YEAST / MOULDS

68 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

68 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J+10
Nb of laboratories	16	16	15	7	6	4	3	1

RESUSCITATION'S CONDITIONS

8 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 **18.5 min** with a standard deviation of 10.6 min. The minimum duration indicated is 1.0 min and the maximum one is 45.0 min.

- TEMPERATURE

60 laboratories specified it.

The average temperature is **22.2°C** with a standard deviation of 4.4°C. The minimum temperature indicated is 8.0°C and the maximum one 47.0°C.

Method	Nb laboratories
NF V08-059	39
→ *NM 08.0.123* [(1)]	5
AFNOR BKR 23/11-12/18	11
ISO / NF ISO 21527-1	4
AFNOR 3M 01/13-07/14	3
AOAC RI 041001	1
NM ISO 21527-1	1
Other	4

Culture medium	Nb laboratories
YGC	34
Symphony	11
Chloramphenicol glucose agar	10
OGA	4
Petrifilm	3
DRBC	3
TEMPO YM	2
Other	1

[(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	39
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	19
Pour	48
Transfer Tempo filler ®	1

Incubation temperature	Nb laboratories
25°C	67
30°C	1

Incubation duration	Nb laboratories
120 h	49
69 - 72 h	15
89 - 96 h	3
54 h	1

2.9. YEAST

65 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
Nb of laboratories	11	15	15	8	11	2	2

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

53 laboratories specified it.

The average duration is **20.8 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.

- TEMPERATURE

53 laboratories specified it.

The average temperature is **21.7°C** with a standard deviation of 2.4°C. The minimum temperature indicated is 18.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
NF V08-059	32
→ *NM 08.0.123* [(1)]	9
AFNOR BKR 23/11-12/18	7
ISO / NF EN ISO 21527-1	5
AFNOR 3M 01/13-07/14	5
NM ISO 21527-1	2
Other	5

Culture medium	Nb laboratories
YGC	30
Chloramphenicol glucose agar	10
Symphony	8
Petrifilm	5
DRBC	5
OGA	3
Other	4

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

Preparation	Nb laboratories
Home made	17
Ready to use not pre-poured	41
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	20
Pour	43

Incubation temperature	Nb laboratories
25°C	62
20°C	3

Incubation duration	Nb laboratories
120 - 125 h	48
72 h	15
96 h	2

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2.10. MOULDS

65 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
Nb of laboratories	11	15	15	8	11	2	2

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

53 laboratories specified it.

The average duration is **20.8 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.

- TEMPERATURE

53 laboratories specified it.

The average temperature is **21.7°C** with a standard deviation of 2.4°C. The minimum temperature indicated is 18.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
NF V08-059	32
→ *NM 08.0.123* [(1)]	9
AFNOR BKR 23/11-12/18	7
ISO / NF EN ISO 21527-1	5
AFNOR 3M 01/13-07/14	5
NM ISO 21527-1	2
Other	5

Culture medium	Nb laboratories
YGC	30
Chloramphenicol glucose agar	10
Symphony	8
Petrifilm	5
DRBC	5
OGA	3
Other	4

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

Preparation	Nb laboratories
Home made	17
Ready to use not pre-poured	41
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	20
Pour	43

Incubation temperature	Nb laboratories
25°C	62
20°C	3

Incubation duration	Nb laboratories
120 - 125 h	48
72 h	15
96 h	2

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 (preservation temperature, preparation of initial suspension and 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, m_{pt}, obtained with algorithm A from the standard NF ISO 13528 applied to all laboratories results included in the statistical analysis.
When groups are constituted, each one is characterized by its own contamination’s assigned value.
The assigned value uncertainty is calculated with the following formula :

$$u(Xpt) = 1{,}25 \times \frac{\sigma pt}{\sqrt{p}}$$

with σ_{pt}, robust standard deviation (standard deviation for proficiency assessment) and p, number of laboratories.

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).

Z-score values are proposed with 3 significant figures.

The standard NF ISO 13528 specifies that:
- |z| ≤ 2,0 is considered as satisfactory (acceptable),
- 2,0 < |z| < 3,0 is considered as a warning signal (questionable),
- |z| ≥ 3,0 is considered as an action signal (or unacceptable).

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	114
Assigned value of the contamination (log cfu/g)	4.947
Uncertainty of assigned value (log cfu/g)	0.0252
Standard deviation for proficiency assessment (log cfu/g)	0.2150

3.2. PSEUDOMONAS

A significant “effect” of the culture medium 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	24	52
Assigned value of the contamination (log cfu/g)	4.084	4.494
Uncertainty of assigned value (log cfu/g)	0.0354	0.0425
Standard deviation for proficiency assessment (log cfu/g)	0.1386	0.2449

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)	3.658
Uncertainty of assigned value (log cfu/g)	0.0183
Standard deviation for proficiency assessment (log cfu/g)	0.1641

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Number of laboratories included in the statistical analysis	66
Assigned value of the contamination (log cfu/g)	3.903
Uncertainty of assigned value (log cfu/g)	0.0385
Standard deviation for proficiency assessment (log cfu/g)	0.2505

3.5. YEAST

None significant effect of the analysis technique has been highlighted.

Yeast	
Number of laboratories included in the statistical analysis	63
Assigned value of the contamination (log cfu/g)	3.831
Uncertainty of assigned value (log cfu/g)	0.0441
Standard deviation for proficiency assessment (log cfu/g)	0.2797

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3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

Moulds	
Number of laboratories included in the statistical analysis	63
Assigned value of the contamination (log cfu/g)	2.967
Uncertainty of assigned value (log cfu/g)	0.0401
Standard deviation for proficiency assessment (log cfu/g)	0.2546

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 NF ISO 13528 §10.8.2.2, explaining the 3 « out of control » situations :

- Just one overtaking of the action limit ($z \leq -3.0$ or $z \geq 3.0$),
- 2 consecutives z scores out of 3 overtaking of the warning limit ($2.0 < z$ or $z < -2.0$),
- 6 consecutives z scores either positive or negative.