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PROFICIENCY TEST « RAEMA »

SCHEME N° 77 A
(4th DECEMBER 2023)
GENERAL REPORT

« The general report is public, it is available on the Website of ASA, results and informations are anonymous, they di not contain any confidential information »

Report authorised by M. CARLIER(1) and L. ALI-MANDJEE
ASA (Postal address) - 149 rue de Bercy, 75012 PARIS

(1) Coordinator of the proficiency test « RAEMA »

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Table of contents

1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

151 laboratories participated to the 77Ath Gel scheme on 4th December 2023 (J0).
We received **149** answers (98.7%).

1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+10
Nb of laboratories	2	101	23	10	6	2	3	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 5.10^3 cfu/g ;
- one sample included a strain of *Bacillus cereus* at a concentration level of 5.10^4 cfu/g ;
- one sample included a strain of *Penicillium* at a concentration level of 5.10^2 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 7 December (J0+3), 11 December (J0+7) and 18 december 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, Yeast and Moulds. For these parameters, inter-samples standard deviation has been included in the calculation of standard deviation for proficiency assessment (NF 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

1.4. EXECUTION OF ANALYZES

1.4.1 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

149 laboratories (100%) specified it.

The average temperature is **4.1°C** with a standard deviation of 1.8°C. The data -4°C given by one laboratory was not taken into account for this calculation 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

148 laboratories (99.3%) specified it.

The average size is **14.5 g** with a standard deviation of 6.6 g. The data 1.24 and 110 g given by 2 laboratories were not taken into account for this calculation The minimum size indicated is 5 g and the maximum one is 30.0 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

148 laboratories (99.3%) specified it.

145 laboratories (97.3%) prepare the initial suspension with adding diluent to gel.

3 laboratories (2.0%) prepare the initial suspension in another way.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

148 laboratories (99.3%) specified it.

137 laboratories (92.0%) use Buffered Peptone Water for the initial suspension.

9 laboratories (6.0%) use Peptone salt solution for the initial suspension.

2 laboratories (1.3%) used another diluent for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

149 laboratories (100%) specified it.

144 laboratories (96.6%) homogenize their sampling with a Stomacher[ND].

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 7 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

114 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

114 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+8	J0+9	J0+10	J0+11
Nb of laboratories	30	23	17	8	2	15	12	3	2	2

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

99 laboratories specified it.

The average duration is **20.3 min** with a standard deviation of 12.7 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

99 laboratories specified it.

The average temperature is **21.0°C** with a standard deviation of 3.2°C. The data 95°C given by one laboratory was not taken into account for this calculation. The minimum temperature indicated is 4.0°C and the maximum one is 30.0°C.

Method	Nb laboratories
ISO / NF EN ISO 15214	82
NM ISO 15214	12
TEMPO LAB	7
AFNOR 3M 01/19-11/17	7
Other	5

Culture medium	Nb laboratories
MRS pH 5.7	90
MRS pH 6.4	9
TEMPO LAB	7
Petrifilm	7
Other	1

Preparation	Nb laboratories
Home made	28
Ready to use not pre-poured	68
Ready to use, plate, film, card	18

Plating method	Nb laboratories
Surface (agar plate, film)	11
Pour	94
Culture medium for card	7

Incubation temperature	Nb laboratories
30°C	112
37°C	2

Incubation duration	Nb laboratories
69 – 75 h	99
40 - 48 h	15

2.6. PSEUDOMONAS

81 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

81 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	23	19	13	2	2	10	9	2	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

69 laboratories specified it.

The average duration is **20.3 min** with a standard deviation of 12.9 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

69 laboratories specified it.

The average temperature is **21.6°C** with a standard deviation of 4.1°C. The data 95°C given by one laboratory was not taken into account for this calculation. The minimum temperature indicated is 8.0°C and the maximum one is 45.0°C.

Method	Nb laboratories
ISO / NF EN ISO 13720	48
AFNOR BKR 23/09-05/15	24
NM ISO 13720	6
Other	3

Incubation temperature	Nb laboratories
25°C	55
30°C	24
22°C	1
37°C	1

Culture medium	Nb laboratories
CFC	57
Rhapsody agar	24

Incubation duration	Nb laboratories
44 - 48 h	79
72 h	2

Preparation	Nb laboratories
Home made	23
Ready to use not pre-poured	26
Ready to use, plate, film, card	32

Confirmation test	Nb laboratories
None	32
Oxydase	48
Other	1

2.7. BACILLUS CEREUS

120 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

120 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+5	J0+7	J0+8	J+9	J0+10	J0+11
Nb of laboratories	28	27	19	9	2	14	13	5	2	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

102 laboratories specified it.

The average duration is **21.3 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

102 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 3.0°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	47
AFNOR BKR 23/06-02/10	28
AFNOR AES 10/10-07/10	22
NM ISO 7932/A1	9
Microval 2014LR47	7
AFNOR BRD 07/26-03/19	3
Other	4

Culture medium	Nb laboratories
Mossel	59
COMPASS *Bacillus cereus* Agar	28
BACARA	22
TEMPO BC	7
RAPID'B. cereus	3
Other	1

Preparation	Nb laboratories
Home made	20
Ready to use not pre-poured	15
Ready to use, plate, film, card	85

Plating method	Nb laboratories
Surface (agar plate, film)	100
Pour	12
Culture medium for card	7

Incubation temperature	Nb laboratories
30°C	118
37°C	2

Incubation duration	Nb laboratories
21 – 25 h	75
42 – 48 h	42
18 – 20 h	3

Confirmation test	Nb laboratories
None	64
Biochemical (including hemolysis)	50
Other	1

2.8. YEAST / MOULDS

65 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

65 laboratories specified it.

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

RESUSCITATION'S CONDITIONS

7 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 **19.8 min** with a standard deviation of 11.5 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

58 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.9°C. The data 95 and 100°C given by 2 laboratories were not taken into account for this calculation. The minimum temperature indicated is 8.0°C and the maximum one 30.0°C.

Method	Nb laboratories
NF V08-059	36
→ *NM 08.0.123* [(1)]	7
AFNOR BKR 23/11-12/18	13
AFNOR 3M 01/13-07/14	3
ISO / NF ISO 21527-1	2
Other	4

Culture medium	Nb laboratories
YGC	32
Symphony	13
Chloramphenicol glucose agar	9
OGA	4
Petrifilm	3
TEMPO YM	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	21
Ready to use not pre-poured	37
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	44

Incubation temperature	Nb laboratories
25°C	60
20 – 22.5°C	3
30°C	2

Incubation duration	Nb laboratories
115 - 120 h	47
63 - 72 h	15
96 h	2
240 h	1

2.9. YEAST

67 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

67 laboratories specified it.

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

57 laboratories specified it.

The average duration is **22.2 min** with a standard deviation of 13.6 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

57 laboratories specified it.

The average temperature is **21.4°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)]	9
AFNOR BKR 23/11-12/18	10
ISO / NF EN ISO 21527-1	4
AFNOR 3M 01/13-07/14	4
Other	6

Culture medium	Nb laboratories
YGC	32
Chloramphenicol glucose agar	12
Symphony	11
Petrifilm	4
DRBC	3
OGA	1
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	14
Ready to use not pre-poured	44
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	48

Incubation temperature	Nb laboratories
25°C	66
20°C	1

Incubation duration	Nb laboratories
120 h	46
69 - 72 h	19
96 h	1
240 h	1

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2.10. MOULDS

67 laboratories performed the enumeration.

DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

67 laboratories specified it.

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

57 laboratories specified it.

The average duration is **22.2 min** with a standard deviation of 13.6 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

- TEMPERATURE

57 laboratories specified it.

The average temperature is **21.4°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)]	9
AFNOR BKR 23/11-12/18	10
ISO / NF EN ISO 21527-1	4
AFNOR 3M 01/13-07/14	4
Other	6

Culture medium	Nb laboratories
YGC	32
Chloramphenicol glucose agar	12
Symphony	11
Petrifilm	4
DRBC	3
OGA	1
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	14
Ready to use not pre-poured	44
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	48

Incubation temperature	Nb laboratories
25°C	66
20°C	1

Incubation duration	Nb laboratories
120 h	46
70 - 72 h	19
96 h	1
240 h	1

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, 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,
- 2,0 < |z| < 3,0 is considered as a warning signal,
- |z| ≥ 3,0 is considered as an action signal (or not acceptable).

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	107
Assigned value of the contamination (log cfu/g)	5.119
Uncertainty of assigned value (log cfu/g)	0.0241
Standard deviation for proficiency assessment (log cfu/g)	0.1994

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	51
Assigned value of the contamination (log cfu/g)	3.498	3.844
Uncertainty of assigned value (log cfu/g)	0.0474	0.0247
Standard deviation for proficiency assessment (log cfu/g)	0.1859	0.1412

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

Bacillus cereus	
Number of laboratories included in the statistical analysis	113
Assigned value of the contamination (log cfu/g)	4.793
Uncertainty of assigned value (log cfu/g)	0.0202
Standard deviation for proficiency assessment (log cfu/g)	0.1721

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Number of laboratories included in the statistical analysis	63
Assigned value of the contamination (log cfu/g)	3.887
Uncertainty of assigned value (log cfu/g)	0.0449
Standard deviation for proficiency assessment (log cfu/g)	0.2854

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 (NF 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	64
Assigned value of the contamination (log cfu/g)	3.887
Uncertainty of assigned value (log cfu/g)	0.0352
Standard deviation for proficiency assessment (log cfu/g)	0.2252

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 (NF ISO 13528 §B.2.5.a).

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3.6. MOULDS

A significant “effect” of the plating method 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 :

Moulds	
Number of laboratories included in the statistical analysis	64
Assigned value of the contamination (log cfu/g)	2.689
Uncertainty of assigned value (log cfu/g)	0.0387
Standard deviation for proficiency assessment (log cfu/g)	0.2480

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

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.

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