

PROFICIENCY TEST « RAEMA »

SCHEME N° 69A (26 NOVEMBER 2019) GENERAL REPORT



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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

148 laboratories participated to the 69Ath Gel scheme on 26th November 2019 (J0).
We received 147 answers.

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+6	J0+7	J0+8	J0+14
Nb of laboratories	5	116	15	4	2	1	3	1

1.3. INFORMATIONS ABOUT SAMPLE

1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of 1.10^6 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 5.10^3 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.

^(a) Coordinator of the proficiency test « RAEMA »

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 28 November (J0+2), 2 December (J0+6) and 9 December 2019 (J0+13).

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.

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 DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

147 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+5	J0+6	J0+7	J0+8	J0+9	J0+10	J0+13	J0+14
Nb of laboratories	24	32	13	1	2	41	22	8	1	1	1	1

1.4.2 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

147 laboratories specified it. The average temperature is **4.0°C** with a standard deviation of 2.1°C. The minimum temperature indicated is 2°C and the maximum one is 20.8°C.

Remark: Please note that samples must be conserved at 4°C on receipt, before analysis.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF TEST SAMPLE

147 laboratories specified it.

The average size is **13.7 g** with a standard deviation of 6.2 g. The minimum size indicated is 1 g and the maximum one is 25 g.

2.2. HOMOGENIZATION TECHNIQUE

147 laboratories specified it.

141 laboratories homogenize their sampling with a StomacherND. 6 laboratories used another technique. The average duration is **2.2 min** with a standard deviation of 1.0 min. The data 10, 15, 20, 60 and 90 min given by 11 laboratories were not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 3 min.

2.3. LACTIC ACID BACTERIA

112 laboratories performed the enumeration.

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

97 laboratories specified it.

The average duration is **20.6 min** with a standard deviation of 12.1 min. The data 120 min given by two laboratories was not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

97 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 3.4°C. The minimum temperature indicated is 4°C and the maximum one is 37°C.

Method	Nb laboratories
NF EN ISO 15214	84
TEMPO LAB	9
NM ISO 15214	8
AFNOR 3M 01/19-11/17	5
Other	6

Culture medium	Nb laboratories
MRS pH 5.7	97
TEMPO LAB	9
Petrifilm	5
Other	1

Preparation	Nb laboratories
Home made	28
Ready to use not pre-poured	69
Ready to use, plate, film, card	15

Plating method	Nb laboratories
Surface (agar plate, film)	10
Pour	92
Culture medium for card	10

Incubation temperature	Nb laboratories
30°C	108
37°C	3
25°C	

Incubation duration	Nb laboratories
70 – 72.5 h	90
44 - 48 h	20
24 h	1
160 h	1

2.4. PSEUDOMONAS

77 laboratories performed the enumeration.

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

65 laboratories specified it.

The average duration is **19.9 min** with a standard deviation of 11.9 min. The data 120 min given by one laboratory was not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

65 laboratories specified it.

The average temperature is **21.0°C** with a standard deviation of 2.2°C. The data 95°C given by one laboratory was not taken into account for this calculation. The minimum temperature indicated is 9.8°C and the maximum one is 27.0°C.

Method	Nb laboratories
NF EN ISO 13720	54
AFNOR BKR 23/09-05/15	14
NM ISO 13720	4
Other	1

Incubation temperature	Nb laboratories
25°C	60
30°C	16
20°C	1

Culture medium	Nb laboratories
CFC	62
Rhapsody agar	15
Other	0

Incubation duration	Nb laboratories
44 - 48 h	73
40 – 42 h	2
30 h	1
72 h	1

Preparation	Nb laboratories
Home made	19
Ready to use not pre-poured	37
Ready to use, plate, film, card	21

Confirmation test	Nb laboratories
None	28
Oxydase	47
Other	2

2.5. BACILLUS CEREUS

114 laboratories performed the enumeration.

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

98 laboratories specified it.

The average duration is **21.8 min** with a standard deviation of 12.5 min. The data 120 min given by two laboratories was not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

98 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 2.8°C. The minimum temperature indicated is 4°C and the maximum one is 30°C.

Method	Nb laboratories
NF EN ISO 7932	65
AFNOR AES 10/10-07/10	21
AFNOR BKR 23/06-02/10	16
NM ISO 7932	6
Microval 2014LR47	4
Other	2

Culture medium	Nb laboratories
Mossel	69
BACARA	22
COMPASS <i>Bacillus cereus</i> Agar	17
TEMPO BC	5
Other	1

Preparation	Nb laboratories
Home made	18
Ready to use not pre-poured	8
Ready to use, plate, film, card	88

Plating method	Nb laboratories
Surface (agar plate, film)	104
Pour	5
Culture medium for card	4

Incubation temperature	Nb laboratories
30°C	111
37°C	3

Incubation duration	Nb laboratories
20 - 24 h	68
42 - 48 h	44
18 h	2

Confirmation test	Nb laboratories
None	55
Biochemical (including hemolysis)	57
Other	1

Heat treatment before enumeration	Nb laboratories
Yes	0
No	112

2.6. YEAST / MOULDS

57 laboratories performed the enumeration.

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

50 laboratories specified it.

The average duration is **21.6 min** with a standard deviation of 12.2 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

50 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 3.1°C. The data 100°C given by one laboratory was not taken into account for this calculation. The minimum temperature indicated is 8.5°C and the maximum one 30°C.

Method	Nb laboratories
NF V08-059	34
→ NM 08.0.123 ⁽¹⁾	6
AFNOR 3M 01/13-07/14	5
AFNOR BKR 23/11-12/18	3
NF ISO 21527-1	2
AOAC RI 041001	1
NM ISO 21527-1	1
Other	5

Culture medium	Nb laboratories
YGC	35
OGA	7
Petrifilm	5
Symphony	3
TEMPO YM	2
DRBC	1
Other	4

Preparation	Nb laboratories
Home made	22
Ready to use not pre-poured	27
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	14
Pour	41
Culture medium for card	2

Incubation temperature	Nb laboratories
24 - 25°C	53
20 - 22.5°C	2
30°C	2

Incubation duration	Nb laboratories
114 - 120 h	43
65 - 72 h	10
88 - 96 h	4

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

2.7. YEAST

59 laboratories performed the enumeration.

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

48 laboratories specified it.

The average duration is **20.6 min** with a standard deviation of 13.6 min. The data 120 min given by two laboratories was not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

48 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 2.0°C. The minimum temperature indicated is 20°C and the maximum one is 27°C.

Method	Nb laboratories
NF V08-059	34
→ NM 08.0.123 ⁽¹⁾	7
AFNOR 3M 01/13-07/14	6
NF ISO 21527-1	5
AFNOR BKR 23/11-12/18	3
Other	4

Culture medium	Nb laboratories
YGC	37
Petrifilm	6
Symphony	4
DRBC	4
OGA	3
Other	5

Preparation	Nb laboratories
Home made	12
Ready to use not pre-poured	38
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	20
Pour	39
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	56
20 – 22°C	2
30°C	1

Incubation duration	Nb laboratories
120 h	40
72 h	11
96 h	6
48 h	1
167 h	1

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

2.8. MOULDS

59 laboratories performed the enumeration.

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

48 laboratories specified it.

The average duration is **20.6 min** with a standard deviation of 13.6 min. The data 120 min given by two laboratories was not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

48 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 2.0°C. The minimum temperature indicated is 20°C and the maximum one is 27°C.

Method	Nb laboratories
NF V08-059	34
→ NM 08.0.123 ⁽¹⁾	7
AFNOR 3M 01/13-07/14	6
NF EN ISO 21527-1	5
AFNOR BKR 23/11-12/18	3
Other	4

Culture medium	Nb laboratories
YGC	37
Petrifilm	6
Symphony	4
DRBC	4
OGA	3
Other	5

Preparation	Nb laboratories
Home made	12
Ready to use not pre-poured	38
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	20
Pour	39
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	56
20 – 22°C	2
30°C	1

Incubation duration	Nb laboratories
120 h	40
72 h	11
96 h	6
48 h	1
167 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	
Assigned value of the contamination (log CFU/g)	6.156
Uncertainty of assigned value (log CFU/g)	0.0308
Standard deviation for proficiency assessment (log CFU/g)	0.2560

3.2. PSEUDOMONAS

A significant “effect” of the size of the test sample and the incubation temperature 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:

Pseudomonas	
Assigned value of the contamination (log CFU/g)	3.500
Uncertainty of assigned value (log CFU/g)	0.0349
Standard deviation for proficiency assessment (log CFU/g)	0.2420

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

Bacillus cereus	
Assigned value of the contamination (log CFU/g)	4.844
Uncertainty of assigned value (log CFU/g)	0.0246
Standard deviation for proficiency assessment (log CFU/g)	0.2044

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Assigned value of the contamination (log CFU/g)	4.203
Uncertainty of assigned value (log CFU/g)	0.0424
Standard deviation for proficiency assessment (log CFU/g)	0.2514

3.5. YEAST

None significant effect of the analysis technique has been highlighted.

Yeast*	
Assigned value of the contamination (log CFU/g)	4.048
Uncertainty of assigned value (log CFU/g)	0.0476
Standard deviation for proficiency assessment (log CFU/g)	0.2875

3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

Moulds	
Assigned value of the contamination (log CFU/g)	3.775
Uncertainty of assigned value (log CFU/g)	0.0265
Standard deviation for proficiency assessment (log CFU/g)	0.1587

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.