

## PROFICIENCY TEST « RAEMA »



### SCHEME N° 81 A (24th NOVEMBER 2025) GENERAL REPORT

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## 1. GENERAL DATA

### 1.1. PARTICIPATING LABORATORIES

144 laboratories participated in the 81A<sup>th</sup> Gel scheme on 24th November 2025 (J0). We received 141 answers (97.9%).

### 1.2. DELIVERY TIME OF THE PARCEL

Delivery time	J0	J0+1	J0+2	J0+3	J0+8	J0+10	J0+14
Nb of laboratories	2	107	22	6	2	1	1

### 1.3. INFORMATIONS ABOUT SAMPLE

#### 1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of  $1.10^6$  cfu/g ;
- one sample included a strain of *Pseudomonas* sp. at a concentration level of  $2.10^4$  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  $6.10^2$  cfu/g and a strain of *Rhodotorula rubra* at a concentration level of  $3.10^3$  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 27 November (J0+3), 1st December (J0+7) and 8 December 2025 (J0+14).

These checks were realized by an external provider accredited by Cofrac for *Bacillus cereus*, lactic bacteria and Yeast/Moulds. The check of *Pseudomonas* was realized by the same provider but not covered by Cofrac accreditation.

Homogeneity of samples has been validated.

Stability of samples has been validated except for Moulds. 11 laboratories have analyzed this parameter during the 2<sup>nd</sup> week and are affected by this stability problem, they have been warned. An analysis of impact has been done; due to the absence of impact on the uncertainty of the assigned value and considering laboratories performances, this impact is low.

#### 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

**139** laboratories (98.6%) specified it.

The average temperature is **4.6°C** with a standard deviation of 2.5°C. The minimum temperature indicated is 2.0°C and the maximum one is 20.4°C.

Remark: Please note that samples must be conserved at  $5\pm3^{\circ}\text{C}$  on receipt, before analysis. They should not be frozen.

## 2. EXPLOITATION OF ANALYSIS REPORT

### 2.1. SIZE OF TEST SAMPLE

**141** laboratories (100%) specified it.

The average size is **14.5 g** with a standard deviation of 6.5 g. The data 1.126 g given by one laboratory was not taken into account for this calculation. The minimum size indicated is 5.0 g and the maximum one is 26.0 g.

### 2.2. PREPARATION OF THE INITIAL SUSPENSION

**140** laboratories (99.3%) specified it.

138 laboratories (97.9%) prepare the initial suspension with adding diluent to gel.

2 laboratories (1.4%) prepare the initial suspension in another way.

### 2.3. DILUENT USED FOR THE INITIAL SUSPENSION

**141** laboratories (100%) specified it.

135 laboratories (95.8%) use Buffered Peptone Water for the initial suspension.

4 laboratories (2.8%) use Peptone salt solution for the initial suspension.

2 laboratories (1.4%) use another diluent for the initial suspension.

### 2.4. HOMOGENIZATION TECHNIQUE

**141** laboratories (100%) specified it.

136 laboratories (96.5%) homogenize their sampling with a Stomacher<sup>ND</sup>.

3 laboratories (2.1%) used a Vortex mixer.

2 laboratories (1.4%) used a manual homogenization.

The average duration is **2.3 min** with a standard deviation of 1.0 min. The data 10, 15 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.

## 2.5. LACTIC ACID BACTERIA

**108** laboratories performed the enumeration.

### TIME / SENDING SAMPLES - BEGINNING OF ANALYZES

**108** laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10	J0+11
Nb of laboratories	20	30	18	8	20	8	1	1	1

### 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

**94** laboratories specified it.

The average duration is **19.7 min** with a standard deviation of 14.0 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

#### - TEMPERATURE

**94** laboratories specified it.

The average temperature is **21.1°C** with a standard deviation of 4.9°C. The minimum temperature indicated is 4.0°C and the maximum one is 47.0°C.

Method	Nb laboratories
ISO / NF EN ISO 15214	79
NM ISO 15214	10
AFNOR 3M 01/19-11/17	7
TEMPO LAB	7
Internal method	2
Other	2

  

Culture medium	Nb laboratories
MRS pH 5.7	86
Neogen® Petrifilm®	7
TEMPO LAB	7
MRS pH 6.4	7

  

Preparation	Nb laboratories
Home made	26
Ready to use not pre-poured	60
Ready to use, plate, film, card	22

Plating method	Nb laboratories
Surface (agar plate, film)	17
Pour	81
Transfer Tempo filler ®	7

  

Incubation temperature	Nb laboratories
30°C	106
37°C	2

  

Incubation duration	Nb laboratories
69 – 73 h	91
42 – 48 h	16
24 h	1

## 2.6. PSEUDOMONAS

**76** laboratories performed the enumeration.

### TIME / SENDING SAMPLES - BEGINNING OF ANALYZES

**76** laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	11	25	18	3	10	6	1	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

**64** laboratories specified it.

The average duration is **18.3 min** with a standard deviation of 11.1 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

#### - TEMPERATURE

**64** laboratories specified it.

The average temperature is **21.5°C** with a standard deviation of 4.4°C. The minimum temperature indicated is 8.0°C and the maximum one is 47.0°C.

Method	Nb laboratories
ISO / NF EN ISO 13720	43
AFNOR BKR 23/09-05/15	24
NM ISO 13720	5
Internal method	3
Other	1

  

Culture medium	Nb laboratories
CFC	51
Rhapsody agar	25

  

Preparation	Nb laboratories
Home made	18
Ready to use not pre-poured	26
Ready to use, plate, film, card	32

Incubation temperature	Nb laboratories
25°C	50
30°C	26

  

Incubation duration	Nb laboratories
44 - 48 h	75
72 h	1

  

Confirmation test	Nb laboratories
None	30
Oxydase	40
MALDI-TOF mass spectrometry	1

## 2.7. BACILLUS CEREUS

**119** laboratories performed the enumeration.

### TIME / SENDING SAMPLES - BEGINNING OF ANALYZES

**119** laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10	J0+17
Nb of laboratories	22	33	24	6	16	12	2	3	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

**101** laboratories specified it.

The average duration is **21.1 min** with a standard deviation of 14.5 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

#### - TEMPERATURE

**101** laboratories specified it.

The average temperature is **21.7°C** with a standard deviation of 5.3°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)	56
AFNOR BKR 23/06-02/10	27
AFNOR AES 10/10-07/10	17
NM ISO 7932 (+A1)	8
Microval 2014LR47	5
AFNOR BRD 07/26-03/19	4
Other	1

  

Culture medium	Nb laboratories
Mossel	63
COMPASS <i>Bacillus cereus</i> Agar	27
BACARA	20
TEMPO BC	5
RAPID'B. cereus	4

  

Preparation	Nb laboratories
Home made	21
Ready to use not pre-poured	12
Ready to use, plate, film, card	86

Plating method	Nb laboratories
Surface (agar plate, film)	102
Pour	9
Transfer Tempo filler ®	5

  

Incubation temperature	Nb laboratories
30°C	119

  

Incubation duration	Nb laboratories
21 – 26 h	76
42 - 48 h	43

  

Confirmation test	Nb laboratories
None	61
Biochemical (including hemolysis)	53
MALDI-TOF mass spectrometry	1

## 2.8. YEAST / MOULDS

**68** laboratories performed the enumeration.

### TIME / SENDING SAMPLES - BEGINNING OF ANALYZES

**67** laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J+11
Nb of laboratories	5	23	17	8	7	5	1	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 **18.9 min** with a standard deviation of 12.4 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

#### - TEMPERATURE

**60** laboratories specified it.

The average temperature is **22.6°C** with a standard deviation of 6.1°C. The minimum temperature indicated is 8.0°C and the maximum one 47.0°C.

Method	Nb laboratories
NF V08-059	41
→ NM 08.0.123 <sup>(1)</sup>	6
AFNOR BKR 23/11-12/18	14
AFNOR 3M 01/13-07/14	3
ISO / NF ISO 21527-1	1
AOAC RI 041001	1
Internal method	1
Other	1

Preparation	Nb laboratories
Home made	23
Ready to use not pre-poured	35
Ready to use, plate, film, card	10
Plating method	Nb laboratories
Surface (agar plate, film)	19
Pour	45
Transfer Tempo filler ®	1

Culture medium	Nb laboratories
YGC	37
Symphony	14
Chloramphenicol glucose agar	9
Neogen® Petrifilm®	3
OGA	2
TEMPO YM	2
DRBC	1

Incubation temperature	Nb laboratories
25°C	65
22 – 22.5°C	2
30°C	1
Incubation duration	Nb laboratories
118 - 120 h	49
69 - 72 h	16
54 h	2
96 h	1

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

## 2.9. YEAST

55 laboratories performed the enumeration.

### TIME / SENDING SAMPLES - BEGINNING OF ANALYZES

55 laboratories specified it.

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

### 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

47 laboratories specified it.

The average duration is **23.4 min** with a standard deviation of 14.5 min. The data 120 min given by one laboratory was not taken into account for this calculation. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

#### - TEMPERATURE

47 laboratories specified it.

The average temperature is **21.5°C** with a standard deviation of 2.9°C. The minimum temperature indicated is 18.0°C and the maximum one is 37.0°C.

Method	Nb laboratories
NF V08-059	28
→ NM 08.0.123 <sup>(1)</sup>	7
AFNOR BKR 23/11-12/18	9
AFNOR 3M 01/13-07/14	5
ISO / NF EN ISO 21527-1	2
Internal method	1
Other	3

  

Culture medium	Nb laboratories
YGC	28
Symphony	9
Chloramphenicol glucose agar	8
Neogen® Petrifilm®	5
OGA	2
DRBC	1
Other	2

Preparation	Nb laboratories
Home made	13
Ready to use not pre-poured	32
Ready to use, plate, film, card	9

  

Plating method	Nb laboratories
Surface (agar plate, film)	14
Pour	38

  

Incubation temperature	Nb laboratories
25°C	53
20-22°C	2

  

Incubation duration	Nb laboratories
120 h	37
69 - 72 h	18

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

## 2.10. MOULDS

**55** laboratories performed the enumeration.

### TIME / SENDING SAMPLES - BEGINNING OF ANALYZES

**55** laboratories specified it.

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

### 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

**47** laboratories specified it.

The average duration is **23.4 min** with a standard deviation of 14.5 min. The data 120 min given by one laboratory was not taken into account for this calculation. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

#### - TEMPERATURE

**47** laboratories specified it.

The average temperature is **21.5°C** with a standard deviation of 2.9°C. The minimum temperature indicated is 18.0°C and the maximum one is 37.0°C.

Method	Nb laboratories
NF V08-059	28
→ NM 08.0.123 <sup>(1)</sup>	7
AFNOR BKR 23/11-12/18	9
AFNOR 3M 01/13-07/14	5
ISO / NF EN ISO 21527-1	2
Internal method	1
Other	3

Preparation	Nb laboratories
Home made	13
Ready to use not pre-poured	32
Ready to use, plate, film, card	9
Plating method	Nb laboratories
Surface (agar plate, film)	14
Pour	38

Culture medium	Nb laboratories
YGC	28
Symphony	9
Chloramphenicol glucose agar	8
Neogen® Petrifilm®	5
OGA	2
DRBC	1
Other	2

Incubation temperature	Nb laboratories
25°C	53
20-22°C	2
Incubation duration	Nb laboratories
120 h	37
69 - 72 h	18

<sup>(1)</sup> 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 (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 :

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

with  $\sigma_{pt}$ , 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  $\sigma_{pt}$  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:

- $|z| \leq 2,0$  is considered as satisfactory (acceptable),
- $2,0 < |z| < 3,0$  is considered as a warning signal (questionable),
- $|z| \geq 3,0$  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.

<b>Lactic acid bacteria</b>	
Number of laboratories included in the statistical analysis	105
Assigned value of the contamination (log cfu/g)	6.008
Uncertainty of assigned value (log cfu/g)	0.0275
Standard deviation for proficiency assessment (log cfu/g)	0.2251
Range of expected satisfactory values (log cfu/g)	[5.558 ; 6.459]

### 3.2. PSEUDOMONAS

None significant effect of the analysis technique has been highlighted.

<b>Pseudomonas</b>	
Number of laboratories included in the statistical analysis	74
Assigned value of the contamination (log cfu/g)	4.384
Uncertainty of assigned value (log cfu/g)	0.0409
Standard deviation for proficiency assessment (log cfu/g)	0.2816
Range of expected satisfactory values (log cfu/g)	[3.821 ; 4.947]

### 3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

<b>Bacillus cereus</b>	
Number of laboratories included in the statistical analysis	116
Assigned value of the contamination (log cfu/g)	4.711
Uncertainty of assigned value (log cfu/g)	0.0202
Standard deviation for proficiency assessment (log cfu/g)	0.1741
Range of expected satisfactory values (log cfu/g)	[4.363 ; 5.059]

### 3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

<b>Yeast - Moulds</b>	
Number of laboratories included in the statistical analysis	65
Assigned value of the contamination (log cfu/g)	3.563
Uncertainty of assigned value (log cfu/g)	0.0326
Standard deviation for proficiency assessment (log cfu/g)	0.2105
Range of expected satisfactory values (log cfu/g)	[3.142 ; 3.984]

### 3.5. YEAST

None significant effect of the analysis technique has been highlighted.

<b>Yeast</b>	
Number of laboratories included in the statistical analysis	54
Assigned value of the contamination (log cfu/g)	3.499
Uncertainty of assigned value (log cfu/g)	0.0384
Standard deviation for proficiency assessment (log cfu/g)	0.2256
Range of expected satisfactory values (log cfu/g)	[3.048 ; 3.950]

### 3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

Moulds	
Number of laboratories included in the statistical analysis	54
Assigned value of the contamination (log cfu/g)	2.806
Uncertainty of assigned value (log cfu/g)	0.0365
Standard deviation for proficiency assessment (log cfu/g)	0.2145
Range of expected satisfactory values (log cfu/g)	[2.377 ; 3.235]

**Comment** : We specify that the stability criterion is unsatisfactory for Moulds enumeration. 11 laboratories have analyzed this parameter during the 2<sup>nd</sup> week and are affected by this stability problem, they have been warned in their individual report.

### 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.