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PROFICIENCY TEST « RAEMA »

réseau d'analyses et d'échanges en microbiologie des aliments

SCHEME N° 70 A
(22th JUNE 2020)
GENERAL REPORT

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Comment :
This edition of 21 september 2021 cancels and replaces the edition of 20 august 2020, on account of an error in the calculation of the value of the standard deviation for proficiency assessment for the parameter Moulds (adjustment following the unsatisfactory homogeneity criterion).

[(1)] Coordinator of the proficiency test « RAEMA »

For any claim, you can use the specific form available on our Website https://association.asa-spv.fr

1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

139 laboratories participated to the 70Ath Gel scheme on 22th June 2020 (J0).
We received **139** answers.

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+4	J0+8	J0+9	J0+10
Nb of laboratories	5	93	30	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 2,5.10^4 cfu/g ;
- one sample included a strain of *Pseudomonas sp.* at a concentration level of 2,5.10^3 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.

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 25 June (J0+3), 29 June (J0+7) and 6 July 2020 (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.

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

139 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+7	J0+8	J0+9	J0+10
Nb of laboratories	35	36	26	10	15	10	4	3

1.4.2 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

139 laboratories specified it. The average temperature is **4.1°C** with a standard deviation of 2.5°C. The minimum temperature indicated is 2.0°C and the maximum one is 25.0°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

139 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 27 g.

2.2. PREPARATION OF THE INITIAL SUSPENSION

137 laboratories specified it.
136 laboratories prepare the initial suspension with adding diluent to gel.
1 laboratory prepares the initial suspension in another way.

2.3. DILUENT USED FOR THE INITIAL SUSPENSION

This data has been added to have all needed elements for this stage.
136 laboratories specified it.
119 laboratories use Buffered Peptone Water for the initial suspension.
17 laboratories used another diluent for the initial suspension.

2.4. HOMOGENIZATION TECHNIQUE

138 laboratories specified it.
133 laboratories homogenize their sampling with a Stomacher[ND].
5 laboratories used another technique.

The average duration is **2.4 min** with a standard deviation of 1.0 min. The data 10, 15, 20, 30 and 60 min given by 10 laboratories were not taken into account for this calculation. The minimum duration indicated is 1 min and the maximum one is 5 min.

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2.5. LACTIC ACID BACTERIA

105 laboratories performed the enumeration.

RESUSCITATION'S CONDITIONS

13 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

92 laboratories specified it.

The average duration is **18.8 min** with a standard deviation of 10.8 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

92 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	82
AFNOR 3M 01/19-11/17	7
NM ISO 15214	6
TEMPO LAB	4
Other	5

Culture medium	Nb laboratories
MRS pH 5.7	91
Petrifilm	7
TEMPO LAB	4
Other	2

Preparation	Nb laboratories
Home made	21
Ready to use not pre-poured	70
Ready to use, plate, film, card	13

Plating method	Nb laboratories
Surface (agar plate, film)	13
Pour	87
Culture medium for card	4

Incubation temperature	Nb laboratories
30°C	104
37°C	1

Incubation duration	Nb laboratories
69 – 73 h	86
44 - 48 h	16
24 h	2
160 h	1

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2.6. PSEUDOMONAS

70 laboratories performed the enumeration.

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

60 laboratories specified it.

The average duration is **19.9 min** with a standard deviation of 12.9 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

60 laboratories specified it.

The average temperature is **20.9°C** with a standard deviation of 2.6°C. The minimum temperature indicated is 6.0°C and the maximum one is 27.0°C.

Method	Nb laboratories
NF EN ISO 13720	43
AFNOR BKR 23/09-05/15	18
NM ISO 13720	3
Other	5

Culture medium	Nb laboratories
CFC	50
Rhapsody agar	19
Other	0

Preparation	Nb laboratories
Home made	15
Ready to use not pre-poured	34
Ready to use, plate, film, card	21

Incubation temperature	Nb laboratories
25°C	53
30°C	17

Incubation duration	Nb laboratories
44 - 48 h	67
41 – 42 h	2
72 h	1

Confirmation test	Nb laboratories
None	28
Oxydase	40
Other	1

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2.7. BACILLUS CEREUS

109 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

93 laboratories specified it.

The average duration is **19.6 min** with a standard deviation of 11.5 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

93 laboratories specified it.

The average temperature is **21.1°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	59
AFNOR BKR 23/06-02/10	23
AFNOR AES 10/10-07/10	17
NM ISO 7932	5
Microval 2014LR47	3
Other	2

Culture medium	Nb laboratories
Mossel	64
COMPASS *Bacillus cereus* Agar	23
BACARA	18
TEMPO BC	3
Other	1

Preparation	Nb laboratories
Home made	15
Ready to use not pre-poured	9
Ready to use, plate, film, card	85

Plating method	Nb laboratories
Surface (agar plate, film)	96
Pour	9
Culture medium for card	3

Incubation temperature	Nb laboratories
30°C	108
37°C	1

Incubation duration	Nb laboratories
19 - 24 h	65
42 - 48 h	44

Confirmation test	Nb laboratories
None	54
Biochemical (including hemolysis)	52
Other	0

Heat traitement before enumaration	Nb laboratories
Yes	0
No	107

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2.8. YEAST / MOULDS

54 laboratories performed the enumeration.

RESUSCITATION'S CONDITIONS

9 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

45 laboratories specified it.

The average duration is **18.2 min** with a standard deviation of 9.7 min. The minimum duration indicated is 1 min and the maximum one is 45 min.

- TEMPERATURE

45 laboratories specified it.

The average temperature is **21.1°C** with a standard deviation of 3.5°C. The minimum temperature indicated is 4°C and the maximum one 30°C.

Method	Nb laboratories
NF V08-059	33
→ *NM 08.0.123* [(1)]	4
AFNOR 3M 01/13-07/14	5
AFNOR BKR 23/11-12/18	4
NF ISO 21527-1	2
AOAC RI 041001	1
NM ISO 21527-1	1
Other	4

Culture medium	Nb laboratories
YGC	32
OGA	7
Petrifilm	5
Symphony	4
TEMPO YM	1
DRBC	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	15
Ready to use not pre-poured	32
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	15
Pour	38
Culture medium for card	1

Incubation temperature	Nb laboratories
24 - 25°C	50
20 - 22°C	2
30°C	2

Incubation duration	Nb laboratories
120 h	36
69 - 72 h	10
88 - 96 h	5
5 h	1
139 h	1
576 h	1

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2.9. YEAST

55 laboratories performed the enumeration.

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 **20.8 min** with a standard deviation of 13.0 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

47 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.1°C. The minimum temperature indicated is 19°C and the maximum one is 27°C.

Method	Nb laboratories
NF V08-059	25
→ *NM 08.0.123* [1]	7
NF EN ISO 21527-1	9
AFNOR BKR 23/11-12/18	5
AFNOR 3M 01/13-07/14	4
NM ISO 21527-1	1
Other	3

Culture medium	Nb laboratories
YGC	28
Symphony	6
OGA	5
DRBC	5
Petrifilm	4
Other	6

Preparation	Nb laboratories
Home made	10
Ready to use not pre-poured	38
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	37
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	53
20 °C	1
30°C	1

Incubation duration	Nb laboratories
120 h	38
69 - 72 h	11
96 h	3
5 h	1
167 h	1
576 h	1

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

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2.10. MOULDS

55 laboratories performed the enumeration.

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 **20.8 min** with a standard deviation of 13.0 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

47 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.1°C. The minimum temperature indicated is 19°C and the maximum one is 27°C.

Method	Nb laboratories
NF V08-059	25
→ *NM 08.0.123* [(1)]	7
NF EN ISO 21527-1	9
AFNOR BKR 23/11-12/18	5
AFNOR 3M 01/13-07/14	4
NM ISO 21527-1	1
Other	3

Culture medium	Nb laboratories
YGC	28
Symphony	6
OGA	5
DRBC	5
Petrifilm	4
Other	6

Preparation	Nb laboratories
Home made	10
Ready to use not pre-poured	38
Ready to use, plate, film, card	7

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	37
Culture medium for card	0

Incubation temperature	Nb laboratories
24 - 25°C	53
20 °C	1
30°C	1

Incubation duration	Nb laboratories
120 h	38
69 - 72 h	11
96 h	3
5 h	1
167 h	1
576 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.

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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)	4.483
Uncertainty of assigned value (log CFU/g)	0.0384
Standard deviation for proficiency assessment (log CFU/g)	0.3119

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
Assigned value of the contamination (log CFU/g)	3.119	3.427
Uncertainty of assigned value (log CFU/g)	0.0438	0.0478
Standard deviation for proficiency assessment (log CFU/g)	0.1526	0.2732

3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

Bacillus cereus	
Assigned value of the contamination (log CFU/g)	4.720
Uncertainty of assigned value (log CFU/g)	0.0266
Standard deviation for proficiency assessment (log CFU/g)	0.2203

3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds	
Assigned value of the contamination (log CFU/g)	4.184
Uncertainty of assigned value (log CFU/g)	0.0582
Standard deviation for proficiency assessment (log CFU/g)	0.3356

3.5. YEAST

None significant effect of the analysis technique has been highlighted.

Yeast	
Assigned value of the contamination (log CFU/g)	3.945
Uncertainty of assigned value (log CFU/g)	0.0687
Standard deviation for proficiency assessment (log CFU/g)	0.4001

3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

Moulds	
Assigned value of the contamination (log CFU/g)	3.676
Uncertainty of assigned value (log CFU/g)	0.0427
Standard deviation for proficiency assessment (log CFU/g)	0.2235

Comment : We specify that the homogenity criterium is unsatisfactory for Moulds enumeration. Inter-samples standard deviation has then be included in the calculation of standard deviation for proficiency assessment.

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.

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