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PROFICIENCY TEST « RAEMA »

SCHEME N° 68A
(21 MAY 2018)
GENERAL REPORT

ACCREDITATION
N°1-1836
SCOPE AVAILABLE ON
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« Only results followed by sign • are covered by accreditation »

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1. GENERAL DATA

1.1. PARTICIPATING LABORATORIES

137 laboratories participated to the 68Ath Gel scheme on 21th May 2019 (J0).
We received **135** answers.

1.2. DELIVERY TIME OF THE PARCEL

Reception	J0	J0+1	J0+2	J0+3	J0+6
Nb of laboratories	6	107	15	6	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 1.10^5 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 1.10^4 cfu/g ;
- one sample included a strain of *Campylobacter jejuni* at a concentration level of 1.10^3 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 23 may (J0+2), 27 may (J0+6) and 3 june 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. The check of *Campylobacter* was realized internally by ASA.

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•

On an experimental basis, enumeration and detection of *Campylobacter* were proposed.

1.4. EXECUTION OF ANALYZES

1.4.1 DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES

135 laboratories specified it.

Analysis time	J0+1	J0+2	J0+3	J0+4	J0+6	J0+7	J0+8	J0+14	J0+16
Nb of laboratories	35	33	20	2	21	18	4	1	1

1.4.2 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

135 laboratories specified it. The average temperature is **4.0°C** with a standard deviation of 1.9°C. The minimum temperature indicated is 2°C and the maximum one is 20.2°C.

2. EXPLOITATION OF ANALYSIS REPORT

2.1. SIZE OF TEST SAMPLE

135 laboratories specified it.

The average size is **14 g** with a standard deviation of 6.4 g. The minimum size indicated is 1 g and the maximum one is 27 g. The data 125 g given by one laboratory was not taken into account for this calculation.

2.2. HOMOGENIZATION TECHNIQUE

135 laboratories specified it.

130 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 1.2 min. The data 15, 20, 30, 60 and 120 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 10 min.

2.3. LACTIC ACID BACTERIA•

102 laboratories performed the enumeration.

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

88 laboratories specified it.

The average duration is **23.1 min** with a standard deviation of 20 min. The minimum duration indicated is 1 min and the maximum one is 120 min.

- TEMPERATURE

88 laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 3.6°C. The minimum temperature indicated is 4°C and the maximum one is 37°C.

Method	Nb laboratories
NF EN ISO 15214	83
→ *NM ISO 15214* (1)	5
TEMPO LAB	6
AFNOR 3M 01/19-11/17	5
Other	3

Culture medium	Nb laboratories
MRS pH 5.7	90
TEMPO LAB	6
Petrifilm	5
Other	1

Preparation	Nb laboratories
Home made	26
Ready to use not pre-poured	61
Ready to use, plate, film, card	15

Plating method	Nb laboratories
Surface (agar plate, film)	12
Pour	84
Culture medium for card	6

Incubation temperature	Nb laboratories
30°C	100
37°C	2

Incubation duration	Nb laboratories
69 - 72 h	85
44 - 48 h	16
168 h	1

(1) *Similar method to NF EN ISO 15214 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

2.4. PSEUDOMONAS•

66 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

55 laboratories specified it.

The average duration is **21.2 min** with a standard deviation of 14.3 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

- TEMPERATURE

55 laboratories specified it.

The average temperature is **20.9°C** with a standard deviation of 2.6°C. The minimum temperature indicated is 7.5°C and the maximum one is 27.0°C.

Method	Nb laboratories
NF EN ISO 13720	48
→ *NM ISO 13720* [(2)]	2
AFNOR BKR 23/09-05/15	15
Other	1

Culture medium	Nb laboratories
CFC	50
Rhapsody agar	15
Other	1

Preparation	Nb laboratories
Home made	18
Ready to use not pre-poured	29
Ready to use, plate, film, card	19

Incubation temperature	Nb laboratories
25°C	50
30°C	15
20°C	1

Incubation duration	Nb laboratories
44 - 48 h	66

Confirmation test	Nb laboratories
None	21
Oxydase	43
Other	1

[(2)] *Similar method to NF EN ISO 13720 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

2.5. BACILLUS CEREUS•

105 laboratories performed the enumeration.

RESUSCITATION'S CONDITIONS

17 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

88 laboratories specified it.

The average duration is **23.3 min** with a standard deviation of 19.9 min. The data 180 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 120 min.

- TEMPERATURE

88 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 3.0°C. The minimum temperature indicated is 4°C and the maximum one is 30°C.

Method	Nb laboratories
NF EN ISO 7932	63
→ *NM ISO 7932* [(3)]	3
AFNOR AES 10/10-07/10	17
AFNOR BKR 23/06-02/10	16
Microval 2014LR47	5
Other	0

Culture medium	Nb laboratories
Mossel	64
BACARA	18
COMPASS *Bacillus cereus* Agar	15
TEMPO BC	5
Other	3

Preparation	Nb laboratories
Home made	16
Ready to use not pre-poured	9
Ready to use, plate, film, card	80

Plating method	Nb laboratories
Surface (agar plate, film)	92
Pour	7
Culture medium for card	5

Incubation temperature	Nb laboratories
30°C	101
37°C	4

Incubation duration	Nb laboratories
20 - 25 h	66
42 - 48 h	39

Confirmation test	Nb laboratories
None	50
Biochemical (including hemolysis)	53
Other	1

Heat traitement before enumaration	Nb laboratories
Yes	0
No	104

[(3)] *Similar method to NF EN ISO 7932 according to ONSSA (Office National de Sécurité Sanitaire des produits Alimentaires).*

2.6. YEAST / MOULDS•

47 laboratories performed the enumeration.

RESUSCITATION'S CONDITIONS

6 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

41 laboratories specified it.

The average duration is **20.8 min** with a standard deviation of 11.5 min. The data 180 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

41 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 3.3°C. The minimum temperature indicated is 7.5°C and the maximum one 30°C.

Method	Nb laboratories
NF V08-059	28
→ *NM 08.0.123* [4]	2
AFNOR 3M 01/13-07/14	5
AFNOR BKR 23/11-12/18	3
AOAC RI 041001	3
NF ISO 21527-1	2
Other	3

Culture medium	Nb laboratories
YGC	25
OGA	6
Petrifilm	5
Symphony	3
TEMPO YM	3
DRBC	1
Other	4

[4] *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	22
Ready to use, plate, film, card	11

Plating method	Nb laboratories
Surface (agar plate, film)	15
Pour	29
Culture medium for card	3

Incubation temperature	Nb laboratories
25 ± 1°C	43
22 - 22.5°C	2
30°C	2

Incubation duration	Nb laboratories
117 - 120 h	32
72 h	11
90 - 96 h	2
360 h	1
168 h	1

2.7. YEAST•

54 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

42 laboratories specified it.

The average duration is **27.9 min** with a standard deviation of 25.7 min. The minimum duration indicated is 1 min and the maximum one is 120 min.

- TEMPERATURE

42 laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.0°C. The data 100°C given by one laboratory was not taken into account for this calculation. The minimum temperature indicated is 20°C and the maximum one is 27°C.

Method	Nb laboratories
NF V08-059	30
→ *NM 08.0.123* [4]	4
NF ISO 21527-1	7
AFNOR 3M 01/13-07/14	6
AFNOR BKR 23/11-12/18	3
Other	4

Culture medium	Nb laboratories
YGC	27
DRBC	6
Petrifilm	6
Symphony	6
OGA	4
Other	5

Preparation	Nb laboratories
Home made	13
Ready to use not pre-poured	33
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	19
Pour	35
Culture medium for card	0

Incubation temperature	Nb laboratories
25 ± 1°C	52
30°C	2

Incubation duration	Nb laboratories
120 h	35
69 - 72 h	14
96 h	5

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

2.8. MOULDS•

53 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

41 laboratories specified it.

The average duration is **28.3 min** with a standard deviation of 25.8 min. The minimum duration indicated is 1 min and the maximum one is 120 min.

- TEMPERATURE

41 laboratories specified it.

The average temperature is **21.4°C** with a standard deviation of 2.1°C. The data 100°C given by one laboratory was not taken into account for this calculation. The minimum temperature indicated is 20°C and the maximum one is 27°C.

Method	Nb laboratories
NF V08-059	30
→ *NM 08.0.123* [(4)]	4
AFNOR 3M 01/13-07/14	6
NF EN ISO 21527-1	6
AFNOR BKR 23/11-12/18	3
Other	4

Culture medium	Nb laboratories
YGC	27
Petrifilm	6
Symphony	6
DRBC	5
OGA	4
Other	5

Preparation	Nb laboratories
Home made	13
Ready to use not pre-poured	32
Ready to use, plate, film, card	8

Plating method	Nb laboratories
Surface (agar plate, film)	18
Pour	35
Culture medium for card	0

Incubation temperature	Nb laboratories
25 ± 1°C	51
30°C	2

Incubation duration	Nb laboratories
120 h	34
69 - 72 h	14
96 h	5

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

2.9. CAMPYLOBACTER - ENUMERATION

31 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

24 laboratories specified it.

The average duration is **20.4 min** with a standard deviation of 15.9 min. The minimum duration indicated is 3 min and the maximum one is 60 min.

- TEMPERATURE

24 laboratories specified it.

The average temperature is **21.1°C** with a standard deviation of 1.7°C. The minimum temperature indicated is 20°C and the maximum one is 25°C.

Method	Nb laboratories
Microval 2009LR28	13
NF EN ISO 10272-2	12
AFNOR BRD 07/25-01/14	3
Microval 2008LR12	2
Microval 2010LR38	1
Other	0

Culture medium	Nb laboratories
CampyFood agar	17
mCCDA	7
Rapid'Campylobacter	3
Brilliance CampyCount agar	3
CASA	1
Other	0

Preparation	Nb laboratories
Home made	3
Ready to use not pre-poured	2
Ready to use, plate, film, card	26

Incubation temperature	Nb laboratories
41 – 42.5°C	31

Incubation duration	Nb laboratories
46 - 48 h	20
42 - 44 h	10
20 h	1

Confirmation test	Nb laboratories
None	7
Morphology / Motility	12
Oxydase	12
Latex agglutination	11
Other	5

2.10. CAMPYLOBACTER - DETECTION

17 laboratories performed the detection.

Method	Nb laboratories
NF EN ISO 10272-1	6
AFNOR BIO 12/30-05/10	6
AFNOR BIO 12/29-05/10	5
Other	0

Enrichment medium	Nb laboratories
CampyFood broth	10
Bolton	4
Preston	3
Other	0

Enrichment temperature	Nb laboratories
41 – 42°C	16
20°C	1

Enrichment duration	Nb laboratories
43 - 48 h	15
24 h	1
30 h	1

Isolation medium	Nb laboratories
CampyFood agar	9
mCCDA	6
CASA	2
Other	4

Preparation	Nb laboratories
Home made	2
Ready to use not pre-poured	0
Ready to use, plate, film, card	15

Isolation temperature	Nb laboratories
41 – 42°C	16

Isolation duration	Nb laboratories
41.5 - 44 h	7
48 h	7
24 h	2

Confirmation test	Nb laboratories
None	3
Morphology / Motility	8
Oxydase	9
Latex agglutination	5
Other	5

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, preparation of the initial suspension, 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).
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) •	5.406•
Uncertainty of assigned value (log CFU/g) •	0.0249•
Standard deviation for proficiency assessment (log CFU/g) •	0.1996•

3.2. PSEUDOMONAS•

A significant "effect" of the confirmation test 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) •	3.985•
Uncertainty of assigned value (log CFU/g) •	0.0382•
Standard deviation for proficiency assessment (log CFU/g) •	0.2445•

3.3. BACILLUS CEREUS•

A significant "effect" of the preparation of the culture medium 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:

Bacillus cereus•	
Assigned value of the contamination (log CFU/g)•	5.372•
Uncertainty of assigned value (log CFU/g)•	0.0225•
Standard deviation for proficiency assessment (log CFU/g) •	0.1825•

3.4. YEAST / MOULDS•

None significant effect of the analysis technique has been highlighted.

Yeast - Moulds•	
Assigned value of the contamination (log CFU/g) •	4.437•
Uncertainty of assigned value (log CFU/g) •	0.0689•
Standard deviation for proficiency assessment (log CFU/g) •	0.3697•

(• Results covered by Cofrac accreditation)

3.5. YEAST•

None significant effect of the analysis technique has been highlighted.

Yeast•	
Assigned value of the contamination (log CFU/g) •	4.426•
Uncertainty of assigned value (log CFU/g) •	0.0615•
Standard deviation for proficiency assessment (log CFU/g) •	0.3548•

3.6. MOULDS•

None significant effect of the analysis technique has been highlighted.

Moulds•	
Assigned value of the contamination (log CFU/g) •	3.053•
Uncertainty of assigned value (log CFU/g) •	0.0257•
Standard deviation for proficiency assessment (log CFU/g) •	0.1468•

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. CAMPYLOBACTER ENUMERATION

Due to a stability problem, this test has been cancelled. No performance has been calculated.

3.8. CAMPYLOBACTER DETECTION

The sample was artificially contaminated.
17 laboratories obtained correct results

3.9. 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.