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# PROFICIENCY TEST « RAEMA »

# SCHEME N° 75 A
# (5th DECEMBER 2022)
# 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 »*

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# 1. GENERAL DATA

## 1.1. PARTICIPATING LABORATORIES

**145 laboratories** participated to the 75A$^{th}$ Gel scheme on 5th December 2022 (J0).
We received **143** answers.

## 1.2. DELIVERY TIME OF THE PARCEL

| Delivery time | J0 | J0+1 | J0+2 | J0+3 | J0+4 | J0+8 | J0+9 | J0+14 |
|---|---|---|---|---|---|---|---|---|
| Nb of laboratories | 2 | 77 | 42 | 7 | 7 | 4 | 2 | 1 |

## 1.3. INFORMATIONS ABOUT SAMPLE

### 1.3.1. NATURE

- one sample included a strain of *Lactobacillus plantarum* at a concentration level of $7.10^5$ 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 $6.10^4$ cfu/g ;
- one sample included a strain of *Penicillium* at a concentration level of $4.10^3$ 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 8 December (J0+3), 12 December (J0+7) and 19 December 2022 (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 *Pseudomonas* and Yeast. For these parameters, inter-samples standard deviation has been included in the calculation of standard deviation for proficiency assessment (ISO 13528 §B.2.5.a).
Stability of samples has been validated except for Moulds. In accordance with the MET50_P1g procedure, the impact has been assessed; there is no impact on participants'results.

### 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



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### 1.4. EXECUTION OF ANALYZES

#### 1.4.1 PRESERVATION TEMPERATURE OF SAMPLES BEFORE ANALYSIS

**143** laboratories specified it.
The average temperature is **3.8°C** with a standard deviation of 0.7°C. The minimum temperature indicated is 2.0°C and the maximum one is 7.0°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

**143** laboratories specified it.
The average size is **14.2 g** with a standard deviation of 7.1 g. The minimum size indicated is 5.0 g and the maximum one is 50.0 g.

### 2.2. PREPARATION OF THE INITIAL SUSPENSION

**142** laboratories specified it.
139 laboratories prepare the initial suspension with adding diluent to gel.
3 laboratories prepare the initial suspension in another way.

### 2.3. DILUENT USED FOR THE INITIAL SUSPENSION

**142** laboratories specified it.
130 laboratories use Buffered Peptone Water for the initial suspension.
9 laboratories use Peptone salt solution for the initial suspension.
3 laboratories used another diluent for the initial suspension.

### 2.4. HOMOGENIZATION TECHNIQUE

**142** laboratories specified it.
134 laboratories homogenize their sampling with a Stomacher$^{ND}$.
4 laboratories used a manual homogenization.
3 laboratories used a Vortex mixer.
1 laboratory used another technique.

The average duration is **2.3 min** with a standard deviation of 1.0 min. The data 15, 20, 30 and 35 min given by 9 laboratories were not taken into account for this calculation. The minimum duration indicated is 0.5 min and the maximum one is 5.0 min.



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## 2.5. LACTIC ACID BACTERIA

**110** laboratories performed the enumeration.

**DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES**

**110** laboratories specified it.

| Analysis time | J0+1 | J0+2 | J0+3 | J0+4 | J0+7 | J0+8 | J0+9 | J0+10 | J0+11 | J0+14 |
|---|---|---|---|---|---|---|---|---|---|---|
| Nb of laboratories | 17 | 31 | 14 | 7 | 26 | 6 | 5 | 1 | 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**

**92** laboratories specified it.

The average duration is **19.8 min** with a standard deviation of 12.5 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

**- TEMPERATURE**

**92** laboratories specified it.

The average temperature is **20.7°C** with a standard deviation of 2.7°C. The minimum temperature indicated is 4.0°C and the maximum one is 27.0°C.

The data 100 min given by 1 laboratory was not taken into account for this calculation.

| Method | Nb laboratories |
|---|---|
| ISO / NF EN ISO 15214 | 79 |
| NM ISO 15214 | 11 |
| TEMPO LAB | 8 |
| AFNOR 3M 01/19-11/17 | 7 |
| Other | 4 |

| Culture medium | Nb laboratories |
|---|---|
| MRS pH 5.7 | 88 |
| TEMPO LAB | 8 |
| Petrifilm | 7 |
| MRS pH 6.4 | 7 |
| Other | 0 |

| Preparation | Nb laboratories |
|---|---|
| Home made | 24 |
| Ready to use not pre-poured | 69 |
| Ready to use, plate, film, card | 17 |

| Plating method | Nb laboratories |
|---|---|
| Surface (agar plate, film) | 16 |
| Pour | 86 |
| Culture medium for card | 8 |

| Incubation temperature | Nb laboratories |
|---|---|
| 30°C | 108 |
| 37°C | 2 |

| Incubation duration | Nb laboratories |
|---|---|
| 69 – 72 h | 91 |
| 41 – 48.5 h | 18 |
| 96 h | 1 |



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## 2.6. PSEUDOMONAS

**76** laboratories performed the enumeration.

**DELIVERY TIME OF 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 | J0+11 | J0+14 |
|---|---|---|---|---|---|---|---|---|---|---|
| Nb of laboratories | 12 | 27 | 11 | 2 | 13 | 6 | 1 | 1 | 2 | 1 |

**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**

**63** laboratories specified it.

The average duration is **18.5 min** with a standard deviation of 11.3 min. The minimum duration indicated is 1 min and the maximum one is 60 min.

**- TEMPERATURE**

**63** laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 3.0°C. The minimum temperature indicated is 10.3°C and the maximum one is 37.0°C.

The data 100 min given by 1 laboratory was not taken into account for this calculation.

| Method | Nb laboratories |
|---|---|
| ISO / NF EN ISO 13720 | 48 |
| AFNOR BKR 23/09-05/15 | 18 |
| NM ISO 13720 | 7 |
| Other | 3 |

| Culture medium | Nb laboratories |
|---|---|
| CFC | 56 |
| Rhapsody agar | 18 |
| Other | 0 |

| Preparation | Nb laboratories |
|---|---|
| Home made | 21 |
| Ready to use not pre-poured | 29 |
| Ready to use, plate, film, card | 26 |

| Incubation temperature | Nb laboratories |
|---|---|
| 25°C | 57 |
| 30°C | 17 |
| 22-23°C | 2 |

| Incubation duration | Nb laboratories |
|---|---|
| 44 - 48 h | 72 |
| 41 – 43 h | 2 |
| 72 h | 2 |

| Confirmation test | Nb laboratories |
|---|---|
| None | 30 |
| Oxydase | 44 |
| Other | 1 |



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## 2.7. BACILLUS CEREUS

**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+7 | J0+8 | J0+9 | J0+10 | J+11 | J0+14 |
|---|---|---|---|---|---|---|---|---|---|---|
| Nb of laboratories | 15 | 36 | 15 | 4 | 28 | 8 | 4 | 2 | 1 | 1 |

**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 **21.0 min** with a standard deviation of 13.5 min. The minimum duration indicated is 1.0 min and the maximum one is 60.0 min.

**- TEMPERATURE**

**98** laboratories specified it.

The average temperature is **21.2°C** with a standard deviation of 2.9°C. The minimum temperature indicated is 4°C and the maximum one is 30°C.

The data 100 min given by 1 laboratory was not taken into account for this calculation.

| Method | Nb laboratories |
|---|---|
| ISO / NF EN ISO 7932/A1 | 49 |
| AFNOR BKR 23/06-02/10 | 25 |
| AFNOR AES 10/10-07/10 | 19 |
| NM ISO 7932 | 11 |
| Microval 2014LR47 | 5 |
| AFNOR BRD 07/26-03/19 | 2 |
| Other | 1 |

| Culture medium | Nb laboratories |
|---|---|
| Mossel | 61 |
| COMPASS *Bacillus cereus* Agar | 24 |
| BACARA | 20 |
| TEMPO BC | 5 |
| RAPID'B. cereus | 2 |
| Other | 1 |

| Preparation | Nb laboratories |
|---|---|
| Home made | 23 |
| Ready to use not pre-poured | 13 |
| Ready to use, plate, film, card | 78 |

| Plating method | Nb laboratories |
|---|---|
| Surface (agar plate, film) | 94 |
| Pour | 11 |
| Culture medium for card | 5 |

| Incubation temperature | Nb laboratories |
|---|---|
| 30°C | 113 |
| 37°C | 1 |

| Incubation duration | Nb laboratories |
|---|---|
| 21 – 24.5 h | 66 |
| 45 – 48 h | 44 |
| 39 – 41 h | 2 |
| 18 h | 2 |

| Confirmation test | Nb laboratories |
|---|---|
| None | 57 |
| Biochemical (including hemolysis) | 51 |
| Other | 1 |

## 2.8. YEAST / MOULDS

**60** laboratories performed the enumeration.

**DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES**

**60** laboratories specified it.

| Analysis time | J0+1 | J0+2 | J0+3 | J0+4 | J0+7 | J0+8 | J0+9 | J0+14 |
|---|---|---|---|---|---|---|---|---|
| Nb of laboratories | 9 | 18 | 10 | 6 | 11 | 4 | 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**

**52** laboratories specified it.

The average duration is **19.2 min** with a standard deviation of 10.6 min. The minimum duration indicated is 1.0 min and the maximum one is 45.0 min.

**- TEMPERATURE**

**52** laboratories specified it.

The average temperature is **21.5°C** with a standard deviation of 3.3°C. The minimum temperature indicated is 8.0°C and the maximum one 30.0°C.

The data 100 min given by 2 laboratories was not taken into account for this calculation.

| Method | Nb laboratories |
|---|---|
| NF V08-059 | 35 |
| → *NM 08.0.123* [(1)] | 4 |
| AFNOR BKR 23/11-12/18 | 10 |
| AFNOR 3M 01/13-07/14 | 4 |
| ISO / NF ISO 21527-1 | 3 |
| NM ISO 21527-1 | 2 |
| Other | 2 |

| Culture medium | Nb laboratories |
|---|---|
| YGC | 32 |
| Symphony | 11 |
| Chloramphenicol glucose agar | 6 |
| OGA | 4 |
| Petrifilm | 4 |
| DRBC | 1 |
| Other | 1 |

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

| Preparation | Nb laboratories |
|---|---|
| Home made | 20 |
| Ready to use not pre-poured | 32 |
| Ready to use, plate, film, card | 8 |

| Plating method | Nb laboratories |
|---|---|
| Surface (agar plate, film) | 18 |
| Pour | 42 |
| Culture medium for card | 0 |

| Incubation temperature | Nb laboratories |
|---|---|
| 23 - 25°C | 55 |
| 30°C | 3 |
| 20 - 22°C | 2 |

| Incubation duration | Nb laboratories |
|---|---|
| 112 - 120 h | 40 |
| 70 - 72 h | 17 |
| 96 h | 2 |
| Other | 1 |



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## 2.9. YEAST

**62** laboratories performed the enumeration.

**DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES**

**61** 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 | 9 | 16 | 7 | 6 | 14 | 5 | 2 | 1 | 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**

**52** laboratories specified it.

The average duration is **22.8 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**

**52** laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.4°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)] | 6 |
| AFNOR BKR 23/11-12/18 | 10 |
| ISO / NF EN ISO 21527-1 | 6 |
| AFNOR 3M 01/13-07/14 | 3 |
| NM ISO 21527-1 | 1 |
| Other | 2 |

| Culture medium | Nb laboratories |
|---|---|
| YGC | 25 |
| Symphony | 12 |
| Chloramphenicol glucose agar | 11 |
| DRBC | 5 |
| OGA | 5 |
| Petrifilm | 3 |
| Other | 1 |

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

| Preparation | Nb laboratories |
|---|---|
| Home made | 13 |
| Ready to use not pre-poured | 41 |
| Ready to use, plate, film, card | 8 |

| Plating method | Nb laboratories |
|---|---|
| Surface (agar plate, film) | 17 |
| Pour | 41 |
| Culture medium for card | 0 |

| Incubation temperature | Nb laboratories |
|---|---|
| 23 - 25°C | 58 |
| 20 - 22°C | 3 |
| 30°C | 1 |

| Incubation duration | Nb laboratories |
|---|---|
| 120 h | 36 |
| 70 - 72 h | 17 |
| 90 - 96 h | 8 |
| Other | 1 |

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## 2.10. MOULDS

**62** laboratories performed the enumeration.

**DELIVERY TIME OF SAMPLES / BEGINNING OF ANALYZES**

**61** 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 | 9 | 16 | 7 | 6 | 14 | 5 | 2 | 1 | 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**

**52** laboratories specified it.

The average duration is **22.8 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**

**52** laboratories specified it.

The average temperature is **21.3°C** with a standard deviation of 2.4°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)] | 6 |
| AFNOR BKR 23/11-12/18 | 10 |
| ISO / NF EN ISO 21527-1 | 6 |
| AFNOR 3M 01/13-07/14 | 3 |
| NM ISO 21527-1 | 1 |
| Other | 2 |

| Culture medium | Nb laboratories |
|---|---|
| YGC | 25 |
| Symphony | 12 |
| Chloramphenicol glucose agar | 11 |
| DRBC | 5 |
| OGA | 5 |
| Petrifilm | 3 |
| Other | 1 |

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

| Preparation | Nb laboratories |
|---|---|
| Home made | 13 |
| Ready to use not pre-poured | 41 |
| Ready to use, plate, film, card | 8 |

| Plating method | Nb laboratories |
|---|---|
| Surface (agar plate, film) | 17 |
| Pour | 41 |
| Culture medium for card | 0 |

| Incubation temperature | Nb laboratories |
|---|---|
| 23 - 25°C | 58 |
| 20 - 22°C | 3 |
| 30°C | 1 |

| Incubation duration | Nb laboratories |
|---|---|
| 120 - 125 h | 36 |
| 70 - 72 h | 17 |
| 96 h | 8 |
| Other | 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, $X_{pt}$, 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 $\sigma_{pt}$ 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

A significant “effect” of the culture medium, manufacturer, plating method and preparation 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 :

| **Lactic acid bacteria** | |
|---|---|
| Number of laboratories included in the statistical analysis | 103 |
| Assigned value of the contamination (log cfu/g) | 5.904 |
| Uncertainty of assigned value (log cfu/g) | 0.0410 |
| Standard deviation for proficiency assessment (log cfu/g) | 0.3329 |

### 3.2. PSEUDOMONAS

None significant effect of the analysis technique has been highlighted.

| ***Pseudomonas*** | |
|---|---|
| Number of laboratories included in the statistical analysis | 71 |
| Assigned value of the contamination (log cfu/g) | 3.954 |
| Uncertainty of assigned value (log cfu/g) | 0.0660 |
| Standard deviation for proficiency assessment (log cfu/g) | 0.4448 |

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

### 3.3. BACILLUS CEREUS

None significant effect of the analysis technique has been highlighted.

| ***Bacillus cereus*** | |
|---|---|
| Number of laboratories included in the statistical analysis | 109 |
| Assigned value of the contamination (log cfu/g) | 4.821 |
| Uncertainty of assigned value (log cfu/g) | 0.0309 |
| Standard deviation for proficiency assessment (log cfu/g) | 0.2581 |



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### 3.4. YEAST / MOULDS

None significant effect of the analysis technique has been highlighted.

| Yeast - Moulds | |
|---|---|
| Number of laboratories included in the statistical analysis | 57 |
| Assigned value of the contamination (log cfu/g) | 4.009 |
| Uncertainty of assigned value (log cfu/g) | 0.0505 |
| Standard deviation for proficiency assessment (log cfu/g) | 0.3052 |

### 3.5. YEAST

None significant effect of the analysis technique has been highlighted.

| Yeast | |
|---|---|
| Number of laboratories included in the statistical analysis | 57 |
| Assigned value of the contamination (log cfu/g) | 3.906 |
| Uncertainty of assigned value (log cfu/g) | 0.0569 |
| Standard deviation for proficiency assessment (log cfu/g) | 0.3438 |

**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 (ISO 13528 §B.2.5.a).

### 3.6. MOULDS

None significant effect of the analysis technique has been highlighted.

| Moulds | |
|---|---|
| Number of laboratories included in the statistical analysis | 57 |
| Assigned value of the contamination (log cfu/g) | 3.601 |
| Uncertainty of assigned value (log cfu/g) | 0.0245 |
| Standard deviation for proficiency assessment (log cfu/g) | 0.1479 |

### 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 either positive or negative.