**Alternative methods for agribusiness**
**Analytical performances certified**

# VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYTICAL METHOD ACCORDING TO STANDARD EN ISO 16140: 2003

**Certificate No.: BKR 23/06 – 02/10**

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| **Validation date:** | **05.02.2010** |
| **Renewal date:** | **28.11.2013** |
| **End of validity:** | **05.02.2018** |

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is hereby authorized to refer to this **NF VALIDATION certificate** for the following alternative quantitative analysis method:

## COMPASS® *Bacillus cereus* Agar

### Validated for the enumeration of presumptive *Bacillus cereus*

Protocol reference: BM126/E/2007-10 : 9

**SCOPE**

All human food products and animal feeding stuffs.

**RESTRICTIONS**

None.

**REFERENCE METHOD**

**EN ISO 7932** (July 2005) : Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 °C.

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**Florence MÉAUX**

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## PRINCIPLE OF THE METHOD

COMPASS® *Bacillus cereus* Agar method for the enumeration of persumtive *Bacillus cereus* strains is based on a chromogenic medium which allows the enumeration of spores and vegetative forms of presumptive *Bacillus cereus*. Caracteristic colonies appear green on the plate and grow after 24 hours on incubation at 30°C (±1°C).

Both inoculation protocols (surface and pour-plate) have been validated in the scope of NF VALIDATION.

In the context of NF VALIDATION, in case of doubt on the characteristic aspect of colonies, a confirmation step may be performed according to the haemolysis test described in ISO 7932 (one colony per plate).

**Note (History of validation)**: In November 2013, the certification of COMPASS *Bacillus cereus* Agar was renewed without perfoming additional validation study. Nor the alternative method, nor the reference method, nor the validation protocol changed since the previous validation study. At the same time, a new dehydrated format has been validated without needs to perform additional tests.

## LINEARITY AND relative ACCURACY

**Comparison of performances of the alternative method and the reference method**

### Linearity study:

Tests were performed in 2009 on the 5 "food product/strain" combinations and for the food categories given in the table below.

The samples were analyzed **in duplicate** with each of the **two methods**, at the five following artificial contamination levels: 100, 500, 1 000, 5 000, 10 000 CFU/g. Both inoculation protocols (surface and pour-plate) were tested.

The following results were obtained, by surface inoculation:

| Food category | Food product/strain pair | Regression line |
|---|---|---|
| Meat and seafood products | Paté de campagne / *Bacillus cereus* 35 | Y = 1.030X - 0.107 |
| Dairy products | Milk powder / *Bacillus cereus* Ad 420 | Y = 0.883X + 0.319 |
| Egg products | Fresh pasta / *Bacillus weihenstephanensis* Ad 780 | Y = 0.945X + 0.147 |
| Vegetables | Puréed vegetables / *Bacillus mycoïdes* Ad 761 | Y = 0.923X + 0.247 |
| Animal feeding stuffs | Biscuits for dog / *Bacillus cereus* 29 | Y = 1.072X - 0.266 |

y = log(N alternative method)
x = log(N reference method)

The following results were obtained, by pour-plate inoculation:

| Food category | Food product/strain pair | Regression line |
|---|---|---|
| Meat and seafood products | Paté de campagne / *Bacillus cereus* 35 | Y = 0.986X - 0.031 |
| Dairy products | Milk powder / *Bacillus cereus* Ad 420 | Y = 0.881X + 0.287 |
| Egg products | Fresh pasta / *Bacillus weihenstephanensis* Ad 780 | Y = 0.915X + 0.161 |
| Vegetables | Puréed vegetables / *Bacillus mycoïdes* Ad 761 | Y = 0.950X + 0.041 |
| Animal feeding stuffs | Biscuits for dog / *Bacillus cereus* 29 | Y = 1.000X – 0.089 |

y = log(N alternative method)
x = log(N reference method)

## Accuracy study:

Tests were performed in 2009. The statistical interpretation was conducted on 86 results for surface inoculation (including 62 artificially contaminated samples) and on 83 results for pour-plate inoculation (including 59 artificially contaminated samples).

The samples represented the following major food categories:

- Meat and seafood products
- Dairy products
- Egg products
- Vegetables
- Animal feeding stuffs

The samples were analyzed **in duplicate** with each of the **two methods**.

As an indication, the contamination (concentration) ranges were as follows:

| Food category | Contamination range (in log CFU/g) | |
|---|---|---|
| | Surface inoculation | Pour-plate inoculation |
| Meat and seafood | 1.48 to 5.23 | 1.54 to 5.20 |
| Dairy products | 1.48 to 4.30 | 1.48 to 4.30 |
| Egg products | 1.60 to 6.79 | 1.90 to 6.79 |
| Vegetables | 1.70 to 5.77 | 1.43 to 5.72 |
| Animal feeding stuffs | 1.48 to 3.74 | 1.48 to 3.74 |

The equation of the regression line between the alternative method and the reference method, for all categories combined, and for each protocol of inoculation, is as follows:

| **Surface inoculation:** | **Pour-plate inoculation:** |
|---|---|
| Y = 0.992X - 0.084 | Y = 0.983X - 0.068 |

y = log(N alternative method)
x = log(N reference method)

The repeatability standard deviations for both methods and the bias between the two methods were determined according to the method of calculation used for the interlaboratory study (see sections 6.3.5 and 6.3.6 of the standard EN ISO 16140/A1). These results provide additional information for the accuracy criterion.

The results were as follows:

| | ***Bias D*** (average of individual bias) | **Repeatability standard deviation (in log)** | |
|---|---|---|---|
| | | Alternative method | Reference method |
| **Surface inoculation** | - 0.083 | 0.084 | 0.84 |
| **Pour-plate inoculation** | - 0.115 | 0.073 | 0.084 |

NB: Limit of repeatability $r = 2.8\ S_r$, with $S_r$: repeatability standard deviation

***Conclusion for linearity and relative accuracy:***

Studies of linearity and of repeatability show that the results obtained with the alternative method are comparable to those obtained with the reference method. The bias between the two methods is low. The repetability of the alternative method by inoculation in surface is slightly upper than the repetability of the reference method, but equivalent using pour-plated inoculation protocol.

## SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)
## Use of alternative method only

- 34 strains of *Bacillus cereus* were detected out of 41 tested. A strain of *B. pseudomycoïdes* gave caracteristic colonies by COMPASS *Bacillus cereus* Agar method using the protocol by pour-plate inoculation. Three others strains of *Bacillus pseudomycoïdes* (Ad 765, Ad 766 and DSM 307) did not grow on COMPASS *Bacillus cereus* Agar plate, but gave caracteristic colonies using the reference method. A strain of *B. weihenstephanensis* (Ad 782) (among five tested) has developed giving white colonies. These strains gave caracteristic colonies on COMPASS® *Bacillus cereus* Agar using BPW supplemented with 1 % of sterilized milk.

- The study of 41 strains not belonging to the genus *Bacillus cereus* did not detect the presence of any cross-reaction.

## PRACTICABILITY
## Use of alternative method only

- **Time of response** : **Positive** and **negative** results are obtained with the alternative method in 1 day against to 3 days with the reference method.

- **Time of manipulation** : COMPASS *Bacillus cereus* Agar method allows an important labour saving by reducing the time of implementation.

## INTERLABORATORY STUDY

The interlaboratory study was conducted in 2009 with 14 participating laboratories. The analyses were carried out on samples of crème anglaise, artificially contaminated with des spores de Bacillus cereus at the 4 following levels:

- 0 CFU/g
- 10 – 100 CFU/g
- 100 – 1,000 CFU/g
- 1 000 – 10,000 CFU/g

The laboratories tested, using the **reference method** and **the alternative method** (testing the protocol by surface inoculation), **two replicates** per contamination level.

The results calculated in accordance with EN ISO 16140 /A1standard were the following:

| Contamination level | Number of laboratories giving exploitable results* | Reference method | | Alternative method | | Bias |
|---|---|---|---|---|---|---|
| | | Repeatability standard deviation $S_r$ | Reproducibility standard deviation $S_R$ | Repeatability standard deviation $S_r$ | Reproducibility standard deviation $S_r$ | |
| Level 1 | 14 | 0.046 | 0.101 | 0.091 | 0.116 | - 0.108 |
| Level 2 | 14 | 0.068 | 0.107 | 0.052 | 0.090 | - 0.043 |
| Level 3 | 14 | 0.119 | 0.110 | 0.090 | 0.143 | - 0.045 |

NB: Limit of repeatability $r = 2.8\ S_r$, with $S_r$: repeatability standard deviation
Limit of reproducibility $R = 2.8\ S_R$, with $S_R$: reproducibility standard deviation

***Conclusion***

The interlaboratory study shows that the results obtained with the alternative method are comparable to those obtained with the reference method.

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| Please send any queries concerning the performance of the validated method to AFNOR Certification. |
| You may download a summary document on the preliminary and inter-laboratory studies on www.afnor-validation.com |