

Contacts:

Stéphanie SAMMARTANO
Senior Certification Engineer
stephanie.sammartano@afnor.org
Phone: +33 (0)1 41 62 62 39

Jérôme FAYOL
Operations supervisor
jerome.fayol@afnor.org
Phone: +33 (0)1 41 62 60 63

Ref.: SSO/JFY/NF102/Clients/SOLABIA/
Avis BT_COMPASS Bcereus_2018-05-17_(R2).docx

Subject: NF VALIDATION mark

SOLABIA SAS
Mrs Natacha GENTEN
2 rue de l'industrie
F-60006 Beauvais
FRANCE

La Plaine Saint-Denis, May 17th, 2018

Dear Madam,

Following the positive agreement expressed on May 17th, 2018, by the Technical Board of the NF VALIDATION mark (NF102), in its application to the food industry, I beg to inform you that the **NF VALIDATION certification has been renewed** for the following alternative method:


COMPASS[®] *Bacillus cereus* Agar

Certificate reference No. BKR 23/06-02/10, with end of validity 05th-February-2022

The alternative method has been validated for the enumeration of presumptive *Bacillus cereus* in all human food products (by performing validation assays on a broad range of foods) and animal food products, by comparison to the reference method NF EN ISO 7932 (2005) and following the validation protocol NF EN ISO 16140-2 (2016).

A further letter will mention full conclusions and possible reservations made by the Technical Board. If reservations are mentioned, I ask you to take them into account without any delay.

Yours Sincerely.



Managing Director
Franck LEBEUGLE



Contacts:

Stéphanie SAMMARTANO
Senior certification engineer
stephanie.sammartano@afnor.org
Phone : +33 (0)1 41 62 62 39

Jérôme FAYOL
Operations supervisor
jerome.fayol@afnor.org
Phone : +33 (0)1 41 62 60 63

Ref.: SSO/SSO/NF102/Clients/SOLABIA/
Avis BT_COMPASS B cereus_2017-11-24 (P1).docx

Subject: NF VALIDATION mark

SOLABIA SAS
Mrs Natacha GENTIE
2 rue de l'industrie
F-60006 Beauvais
FRANCE

La Plaine Saint-Denis, November 24th, 2017

Dear Madam,

The NF VALIDATION certificate of the following alternative method:

COMPASS <i>Bacillus cereus</i> Agar	Ref. BKR 23/06-02/10
--	-----------------------------

will expire on February 5th, 2018, before that complete results of the renewal study may be examined by the Technical Board "Agri-Food" of the NF VALIDATION mark (NF102).

Following the positive agreement of the dedicated Technical Board, I declare that you can continue to refer to this certificate till May 31st, 2018.

Yours Sincerely.



Managing Director
Franck LEBEUGLE





**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

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

The Company
(Head office)

Solabia S.A.S.
29 rue Delizy
93698 PANTIN cedex
France

Production site

Biokar Diagnostics
Rue des Quarante Mines
ZAC de Ther, Allonne
B.P. 10245 – 60002 Beauvais Cedex
France

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.

**Managing Director
Florence MÉAUX**

PRINCIPLE OF THE METHOD

COMPASS® *Bacillus cereus* Agar method for the enumeration of presumptive *Bacillus cereus* strains is based on a chromogenic medium which allows the enumeration of spores and vegetative forms of presumptive *Bacillus cereus*. Characteristic 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 performing 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 / <i>Bacillus cereus</i> 35	$Y = 1.030X - 0.107$
Dairy products	Milk powder / <i>Bacillus cereus</i> Ad 420	$Y = 0.883X + 0.319$
Egg products	Fresh pasta / <i>Bacillus weihenstephanensis</i> Ad 780	$Y = 0.945X + 0.147$
Vegetables	Puréed vegetables / <i>Bacillus mycoïdes</i> Ad 761	$Y = 0.923X + 0.247$
Animal feeding stuffs	Biscuits for dog / <i>Bacillus cereus</i> 29	$Y = 1.072X - 0.266$

$y = \log(N \text{ alternative method})$
 $x = \log(N \text{ reference method})$

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

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

$y = \log(N \text{ alternative method})$
 $x = \log(N \text{ reference method})$

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: $Y = 0.992X - 0.084$	Pour-plate inoculation: $Y = 0.983X - 0.068$
---	--

$y = \log(N \text{ alternative method})$

$x = \log(N \text{ reference method})$

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:

	<i>Bias D</i> (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 repeatability of the alternative method by inoculation in surface is slightly upper than the repeatability 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 characteristic 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 characteristic colonies using the reference method. A strain of *B. weihenstephanensis* (Ad 782) (among five tested) has developed giving white colonies. These strains gave characteristic 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 /A1 standard 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.

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