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
Dear Madam,

The NF VALIDATION certificate of the following alternative method:

| COMPASS Bacillus cereus 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,

[Signature]

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
Solabia S.A.S.
(Head office)
29 rue Delizy
93698 PANTIN cedex
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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


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

AFNOR Certification
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www.afnor.org - www.afnor-validation.com
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:

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

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

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

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

y = log(N 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:

<table>
<thead>
<tr>
<th>Food category</th>
<th>Contamination range (in log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface inoculation</td>
</tr>
<tr>
<td>Meat and seafood</td>
<td>1.48 to 5.23</td>
</tr>
<tr>
<td>Dairy products</td>
<td>1.48 to 4.30</td>
</tr>
<tr>
<td>Egg products</td>
<td>1.60 to 6.79</td>
</tr>
<tr>
<td>Vegetables</td>
<td>1.70 to 5.77</td>
</tr>
<tr>
<td>Animal feeding stuffs</td>
<td>1.48 to 3.74</td>
</tr>
</tbody>
</table>

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:
\[ y = 0.993X - 0.068 \]

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

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:

<table>
<thead>
<tr>
<th>Bias ( D ) (average of individual bias)</th>
<th>Repeatability standard deviation (in log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alternative method</td>
</tr>
<tr>
<td>Surface inoculation</td>
<td>- 0.083</td>
</tr>
<tr>
<td>Pour-plate inoculation</td>
<td>- 0.115</td>
</tr>
</tbody>
</table>

NB: Limit of repeatability \( r = 2.8 S_n \) 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. pseudomycoides gave characteristic colonies by COMPASS Bacillus cereus Agar method using the protocol by pour-plate inoculation. Three others strains of Bacillus pseudomycoides (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:

<table>
<thead>
<tr>
<th>Contamination level</th>
<th>Number of laboratories giving exploitable results*</th>
<th>Reference method</th>
<th>Alternative method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>repeatability standard deviation $S_r$</td>
<td>reproducibility standard deviation $S_r$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>repeatable standard deviation $S_r$</td>
<td>reproducible standard deviation $S_r$</td>
</tr>
<tr>
<td>Level 1</td>
<td>14</td>
<td>0.046</td>
<td>0.101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.091</td>
<td>0.116</td>
</tr>
<tr>
<td>Level 2</td>
<td>14</td>
<td>0.068</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.052</td>
<td>0.090</td>
</tr>
<tr>
<td>Level 3</td>
<td>14</td>
<td>0.119</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.090</td>
<td>0.143</td>
</tr>
</tbody>
</table>

NB: Limit of repeatability $r = 2.8 S_r$ with $S_r$: repeatability standard deviation
Limit of reproducibility $R = 2.8 S_{rr}$ with $S_{rr}$: 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