Dear Sir,

Following the positive agreement expressed on November 22nd, 2017, 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:

**RAPID’E.coli 2 - E. coli at 37°C**

Certificate reference No. BRD 07/07-12/04, with end of validity 02nd-December-2020

The alternative method is validated by comparison to the reference method NF ISO 18649-2 (2001) and according to the validation protocol NF EN ISO 16140-2 (2016) for the enumeration at 37°C of β-glucuronidase positive E. coli in all human food products (by performing validation assays on a broad range of foods).

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
La Plaine Saint-Denis, July 4th, 2017

Dear Sir,

The NF VALIDATION certificate of the following analysis method:

<table>
<thead>
<tr>
<th>RAPID’E. COLI 2</th>
<th>Ref. BRD 07/07-12/04</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-glucuronidase-positive E. coli at 37°C</td>
<td></td>
</tr>
</tbody>
</table>

will expire on July 4th, 2017 before that complete results of the renewal study may be examined by the Technical Board "Food microbiology" of the NF VALICATION mark (NF102).

Following the positive agreement of the Technical Board, I declare that you can continue to refer to this certificate till November 24th, 2017.

Yours Sincerely,

[Signature]
Managing Director
Franck LEBEUGLE
Dear Sir,

The NF VALIDATION certificate of the following analysis method:

<table>
<thead>
<tr>
<th>RAPID’E. COLI 2</th>
<th>Ref. BRD 07/07-12/04</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-glucuronidase-positive E. coli at 37°C</td>
<td></td>
</tr>
</tbody>
</table>

will expire on June 2nd, 2017 before that complete results of the renewal study may be examined by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102).

Following the positive agreement of the Technical Board, I declare that you can continue to refer to this certificate till July 4th, 2017.

Yours Sincerely,

[Signature]

Managing Director
Franck LEBEJUGLE
Dear Sir,

The NF VALIDATION certificate of the following analysis method:

<table>
<thead>
<tr>
<th>RAPID'E. COLI 2</th>
<th>Ref. BRD 07/07-12/04</th>
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</thead>
<tbody>
<tr>
<td>β-glucuronidase-positive E. coli at 37°C</td>
<td></td>
</tr>
</tbody>
</table>

will expire on December 2nd, 2016 before that complete results of the renewal study may be examined by the Technical Board "Food microbiology" of the NF VALIDATION mark (NF102).

Following the positive agreement of the Technical Board, I declare that you can continue to refer to this certificate till June 2nd, 2017.

Yours Sincerely.

Managing Director
Franck LEBEUGLE
VALIDATION CERTIFICATE FOR ALTERNATIVE ANALYTICAL METHOD
ACCORDING TO STANDARD EN ISO 16140: 2003

Certificate No.: BRD 07/07 – 12/04

Validation date: 02.12.2004
Renewal dates: 28.11.2008
29.11.2012

The Company (Head office)
BIO-RAD
3, Boulevard Raymond Poincaré
92430 MARNES LA COQUETTE
FRANCE

Production site
BIO-RAD
Route de Cassel
59114 STEENVOORDE
FRANCE

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

RAPID’E. COLI 2
VALIDATED FOR THE ENUMERATION OF β-GLUCURONIDASE-POSITIVE E. COLI AT 37°C

Protocol reference: RAPID’E. coli 2 / Agar – V5

SCOPE
All food products for human consumption.

RESTRICTIONS
None.

REFERENCE METHOD

Managing Director
Florence MÉAUX
PRINCIPLE OF THE METHOD

The principle of the RAPID'E.coli 2 medium is based on the simultaneous detection of two enzyme activities: Beta-β-Glucuronidase (GLUC) and Beta-D-Galactosidase (GAL).

The medium contains two chromogenic substrates:
- one GAL-specific substrate inducing blue coloration of colonies positive for this enzyme.
- one GLUC-specific substrate inducing pink coloration of colonies positive for this enzyme.

Coliforms other than E.coli (GAL+/GLUC-) form blue colonies, E.coli (GAL+/GLUC+) form purple to pink colonies.

NOTE (History of validation)

In November 2012, the validation was renewed without conducting additional validation tests, because nor the the reference method nor the alternative method changed since the last validation study. The interlaboratory study results obtained in 2004 were re-calculated in accordance to EN ISO 16140/A1 standard, without impact on the conclusion of the study.

LINEARITY AND relative ACCURACY
Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2004 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: 10 to 50, 50 to 100, 100 to 500, 500 to 1,000, 1,000 to 10,000 CFU/g.

The following results were obtained:

<table>
<thead>
<tr>
<th>Food category</th>
<th>Food product/strain pair</th>
<th>Regression line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat products</td>
<td>Ground beef/ E.coli source pork kidneys</td>
<td>y = 0.061 + 1.003 x</td>
</tr>
<tr>
<td>Dairy products</td>
<td>Raw milk/ E.coli source raw milk</td>
<td>y = -0.002 + 0.990 x</td>
</tr>
<tr>
<td>Vegetable products</td>
<td>Red cabbage/ E.coli source red cabbage</td>
<td>y = 0.037 + 0.990 x</td>
</tr>
<tr>
<td>Seafood products</td>
<td>Fish filet/ E.coli source flat sausage</td>
<td>y = -0.188 + 1.096 x</td>
</tr>
<tr>
<td>Cakes and pastries</td>
<td>Confectioner's custard/ E.coli source vanilla custard</td>
<td>y = 0.202 + 0.951 x</td>
</tr>
</tbody>
</table>

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

Accuracy study:

Tests were performed in 2004. The statistical interpretation was conducted on 50 results, including 45 naturally contaminated samples and 5 artificially contaminated samples, belonging to the following major food categories:

Meat products, dairy products, vegetable products, seafood products, cakes and pastries.
During the tests, the alternative method was unable to produce a result for 4 samples, unlike the reference method, due to the invasion of the agars by a high level of interfering flora.

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>Meat products</td>
<td>1.0 to 3.1</td>
</tr>
<tr>
<td>Dairy products</td>
<td>0.5 to 4.3</td>
</tr>
<tr>
<td>Vegetable products</td>
<td>1.0 to 3.8</td>
</tr>
<tr>
<td>Seafood products</td>
<td>2.0 to 5.5</td>
</tr>
<tr>
<td>Cakes and pastries</td>
<td>1.5 to 4.5</td>
</tr>
</tbody>
</table>

The equation of the regression line and the correlation coefficient ($r^2$) between the alternative method and the reference method, for all categories combined, are as follows:

$$y = 0.100 + 0.968 \times x$$

$r^2 = 0.984$

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

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

The repeatability standard deviation 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 repeatability standard deviations (in log) obtained for the alternative method and the reference method are as follows:

- **Alternative method**
  - $S_{\text{alt.}} = 0.101$

- **Reference method**
  - $S_{\text{Ref.}} = 0.115$

**NB:** Limit of repeatability $r = 2.8 \times S_{\text{r}}$ with $S_{\text{r}}$: repeatability standard deviation

The bias (in log) between the two methods (alternative method - reference method) is as follows:

$$D = 0.012 \log$$

**Conclusion for linearity and relative accuracy:**

The linearity and accuracy studies demonstrate that the results obtained with the alternative method are comparable to the results obtained with the reference method.

**SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)**

**Use of alternative method only**

- 30 β-glucuronidase-positive *E.coli* strains were detected out of 30 tested.

- The study of 54 non-*E.coli* strains revealed negative reactions for 51 strains (no colonies or non-characteristic colonies) and positive reactions (characteristic appearance) with the 3 following strains: one *Shigella sonnei* strain and 2 *Salmonella arizonae* strains (lactose +).

  These three strains tested with the reference method also produced characteristic (blue) colonies on TBX- medium.
PRACTICABILITY
Use of alternative method only

- **Positive** and **negative** results are obtained in 18 hours to 24 hours with both methods (alternative and reference).
- The only differences between the RAPID’E. coli 2 method and the reference method consist in the medium used and the color of the characteristic colonies.
- RAPID’E.coli 2 medium is used in a single layer, except for products with a very high concentration of interfering flora, and makes it possible to distinguish between *E.coli* and other coliforms.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2004 with 16 participating laboratories. The analyses were carried out on samples of pasteurized milk artificially contaminated with a β-glucuronidase-positive *E.coli* strain isolated from a pastry at the 4 following levels:

- level 0
- level 1: 10 - 100 CFU/ml
- level 2: 100 - 1,000 CFU/ml
- level 3: 1,000 - 10,000 CFU/ml

The laboratories tested, using each of the **two methods**, **two replicates per contamination level**.

The results calculated in accordance with the EN ISO 16140/A1 were the following:

<table>
<thead>
<tr>
<th>Contamination level</th>
<th>Number of samples taken into account*</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>Level 1</td>
<td>40</td>
<td>0.131</td>
<td>0.146</td>
</tr>
<tr>
<td>Level 2</td>
<td>40</td>
<td>0.056</td>
<td>0.139</td>
</tr>
<tr>
<td>Level 3</td>
<td>40</td>
<td>0.115</td>
<td>0.148</td>
</tr>
</tbody>
</table>

* 4 laboratories did not conduct the analysis.
* One laboratory conducted the analysis 48 hours after receipt, i.e. 72 hours after shipment and its results were not taken into account.

**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 collaborative study demonstrates that the results obtained with the alternative method are comparable to the results 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](http://www.afnor-validation.com)