CHROMagar™

The Chromogenic Media Pioneer

CHROMagar™ Listeria numeration Method

VALIDATION REPORT

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PRINCIPLE OF THE METHOD

CHROMagar™ Listeria numeration for enumeration of *Listeria monocytogenes* is a method including a suspension broth and the chromogenic culture media CHROMagar™ Listeria , allowing the specific detection of *Listeria monocytogenes*.

In case of positive result with the enumeration method, confirmation is not necessary when the presence of *Listeria monocytogenes* has already been confirmed by the detection method.

In the other cases, samples identified as positive by the alternative method must be confirmed by one of the following means:

- According to classical tests described in methods standardised by CEN or ISO (including a purification step), starting from CHROMagar™ Listeria.
- Directly from a typical colony well isolated on CHROMagar™ Listeria by spotting the colony onto CHROMagar™ Identification Listeria: *Listeria monocytogenes* displays a mauve colour surrounded by a white opaque halo.

Note : If confirmation has already been done for a same sample during the detection phase with the CHROMagar Listeria method (CHR 21/01 – 12/01), a new confirmation is not necessary after the CHROMagar Listeria enumeration method.
If you do not confirm 5 colonies counting, it can create a risk of making a overestimated result because of the possible presence of typical colonies that would not be *L. monocytogenes*.

In the event of discordant results (presumptive positive with alternative method, non-confirmed by means of options described above) the laboratory must follow the necessary steps to ensure validity of the results obtained.

NOTE (validation history)

In September 2009, the certification was renewed for CHROMagar™ Listeria numeration without complementary assays. Since the previous validation in 2006, the formula of CHROMagar™ Listeria numeration test has not changed, as well as reference method and the EN ISO 16140 protocol.

In October 2013, the certification of the alternative method has been renewed. This one did not change since the last validation study, and also the reference methid. The interlaboratory results obtained in 2006 were re-analysed according to EN ISO 16140/A1, without any impact on the study conclusions.

Tests were done with two plates by dilution for the reference method, and one plate by dilution for the alternative method.

LINEARITY AND relative ACCURACY

Comparison of performances of the alternative method and the reference method

Linearity study:

Tests were performed in 2006 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 and 1 000 to 10 000 CFU/g.

The following results were obtained:

Food category	Food product/strain pair	Regression line
Meat products	Pâté / *L. monocytogenes* 4e	Y = 0.932 X + 0.305
Dairy products	Raw milk / *L. monocytogenes* 1/2a	Y = 1.011 X – 0.004
Vegetal products	Salad / *L. monocytogenes* 1/2a	Y = 1.024 X – 0.051
Seafood products	Smoked salmon / *L. monocytogenes* 1/2b	Y = 1.006 X – 0.031
Environment samples	Industrial water / *L. monocytogenes* 1/2a	Y = 0.967 X + 0.122

Y = log(N alternative method)
X = log(N reference method)

Accuracy study :

Tests were performed in 2006. The statistical interpretation was conducted on 54 results, including 10 naturally contaminated samples and 44 artificially contaminated samples, belonging to the following major food categories:

Meat products, dairy products, vegetal products, seafood products and environmental samples

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)
Meat products	1,70 to 4,15
Dairy products	1,48 to 4,08
Vegetal products	1,60 to 4,00
Seafood products	1,84 to 5,11
Environment samples	1,70 to 5,18

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

Y = 1,008 X – 0,012

Y = log(N alternative method)
X = log(N 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	Reference method
Sr alt. = 0.119	Sr ref. = 0.100

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

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

D = 0,02 if one consider the median value
or **p = 0,01** if one considers the average of individual bias.

Conclusion for linearity and relative accuracy:

Studies of linearity and relative accuracy show that results obtained with the alternative method are similar to those obtained with the reference method.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)
Use of alternative method only

Specificity of the alternative method has been studied in 2001, during the validation of the method CHROMagar™ Listeria (CHR 21/01 – 12/01) for the detection of *Listeria monocytogenes*.

- 50 strains of were detected out of 50 tested.
- The study of 31 strains not belonging to the genus *Listeria monocytogenes* did not detect the presence of cross-reactions.

PRACTICABILITY
Use of alternative method only

- **Time of response**:

- **Positive** results are obtained with the alternative method in 3 days (if confirmation with CHROMagar™ Identification Listeria test), or 4 to 7 days (if confirmation with classical tests), as opposed to 4 to 7 days with the reference method.

- **Negative** results are obtained in 2 days with both alternative method and reference method.

INTER-LABORATORY STUDY

The inter-laboratory study was conducted in 2006 with 12 participating laboratories. The analyses were carried out on samples of milk (obtained by mixing 50% of pasteurized milk with 50% of half skimmed milk), artificially contaminated with a *Listeria monocytogenes 1/2a* strain at the 4 following levels:

- Level 0: < 10 CFU/ml
- Level 1: 50 - 500 CFU/ml
- Level 2: 500 – 5,000 CFU/ml
- Level 3: 5,000 – 50,000 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 standard were the following (for the 3 levels superior to 50 CFU/ml):

Contamination level	Number of samples taken into account*	Reference method		Alternative method		
		Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Bias
Level 1	10	0.048	0.106	0.086	0.086	0.035
Level 2	10	0.025	0.033	0.056	0.077	- 0.019
Level 3	10	0.048	0.048	0.061	0.079	- 0.017

** Two laboratories have been excluded, one for not receiving the sample in time, the other for not following the protocol of analysis.*

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

Inter-laboratory study shows that results obtained with alternative method are comparable to those obtained with reference method.