anses
French agency for food, environmental and occupational health & safety

EURL *Lm*

European Union Reference Laboratory for *Listeria monocytogenes*

Measurement uncertainty for *L. monocytogenes* enumeration:

Trials on influence of sub-sampling test portion

Léna BARRE; Emilie BRASSEUR; Bertrand LOMBARD

WS Listeria monocytogenes, 25-27 March 2015

Measurement uncertainty for *L. monocytogenes* enumeration

1. Context and objectives
2. Previous results obtained
3. M & M
4. Results
5. Conclusion

Context

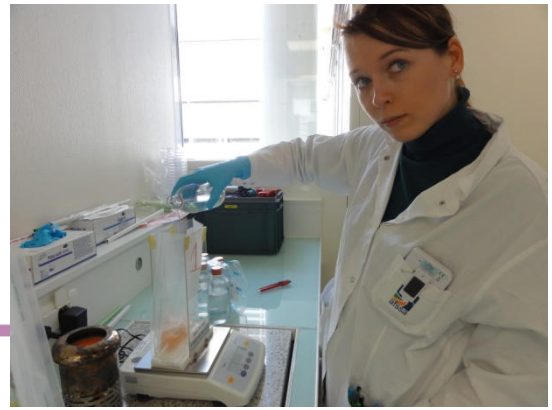
• The reference **method EN ISO 11290-2/A1** is the Standard method for :

ENUMERATION of *Listeria monocytogenes*
(L. monocytogenes, Lm) in food

- EN ISO 6887-1 to 6 on preparation of test samples for microbiological analyses

• The distribution of microorganisms in solid matrices is heterogeneous and complex

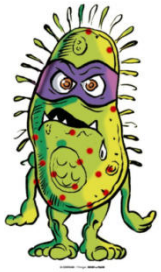
Sub-sampling of test portion
= major source of measurement uncertainty (MU), in particular for solid food matrices



Context

To reduce MU:

To harmonize/specify the sub-sampling procedure /preparation of sample, **test portion** & initial suspension



- **Revision of ISO/TS 19036*/ EN ISO 6887 **series ?**

*** Guidelines for the estimation of measurement uncertainty for quantitative determinations**

**** Preparation of test samples, initial suspension and decimal dilutions for microbiological examination**

Measurement uncertainty for *L. monocytogenes* enumeration

1. Context and objectives
2. Previous results obtained
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Previous results : presented at TERAMO

➢ At first sight, in all samples, we have concluded that the contamination was **heterogeneous** whatever the products were (meat, cheese…) -> results in 2010

➢ According to some assays performed earlier in our lab (Poumeyrol; 2008) and to a bibliographic study (Corry;2010)

✓ For the preparation of the initial suspension, the test portion **should be ≥ 100g**

2013/2014 : Results « 100g/10g »

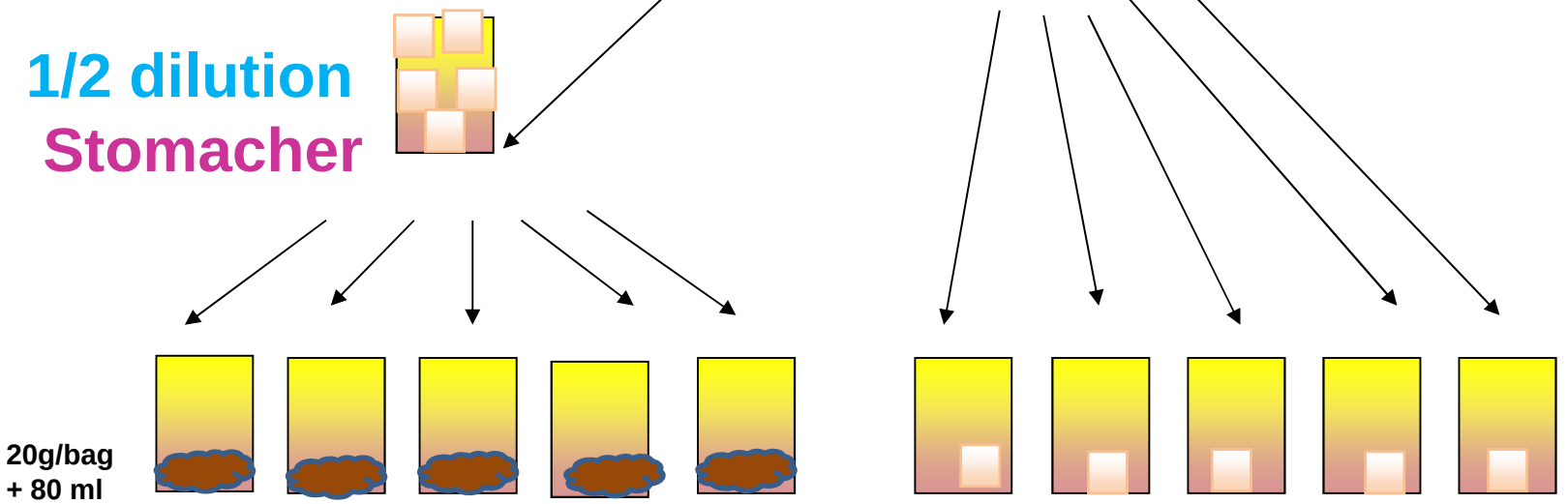
« 100g Method»

« 10g Method »

Naturally contaminated matrix

100g per bag
(5 spots of 20g per bag)

10g (10g in one spot per bag)



1/2 dilution Stomacher

20g/bag + 80 ml

1/5 dilution - Stomacher

Enumeration *Lm* & total flora

1/10 dilution - Stomacher

Enumeration *Lm* and total flora

Conclusion

- ***Lm*** **contamination heterogeneous whatever the matrix is**

Aim : to reduce MU and to obtain realistic results of enumeration

- **Total microflora is not a good indicator of heterogeneity of contamination by *Lm***

- **Results of Lm enumeration depend on preparation of samples / dilution:**
  - **Homogeneization without diluent**
  - **Different sample sizes: 10g/ 100g**

Better MU with 100g

Conclusion

EURL recommandation : 100g-test portion

Teramo :

☹ Difficult to homogeneize 100g !!!!

☹ Expensive …

> Possibility to recommend a 25g-test portion

Standard EN ISO 6887 under revision (SC9 WG8):

- **100g test portion > No way !**
- **Note :**

Using larger test portions will increase the reliability of enumeration test results, particularly for **heterogeneous** contamination in matrices

- **EURL results included in Annex A of EN ISO 6887-1 ?**

Conclusion

- **For technical reason, a 25g-test portion could be recommended**
- **Comparison of MU obtained with 10g/25g test portions**
- **Need of European/NRLs collaboration**

  - **Collaboration EURL *Listeria* / *Staphylococcus aureus***

1. Context and objectives
2. Previous results obtained
3. M & M
4. Results
5. Conclusion

Trials : test portion« 25g/10g »

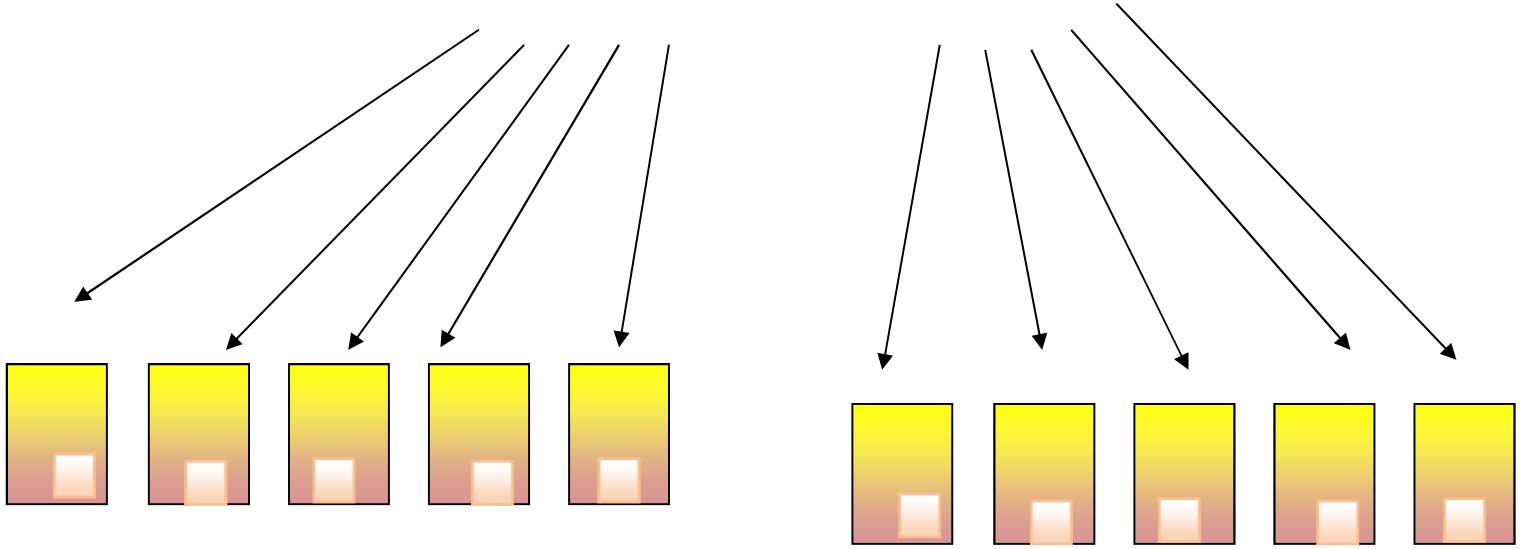
« 25g Method »

« 10g Method »

Naturally contaminated matrix

25 g per bag
(5 spots of 5g per bag)

10g
(~5 spots of 2g per bag)



Dilution 1/10

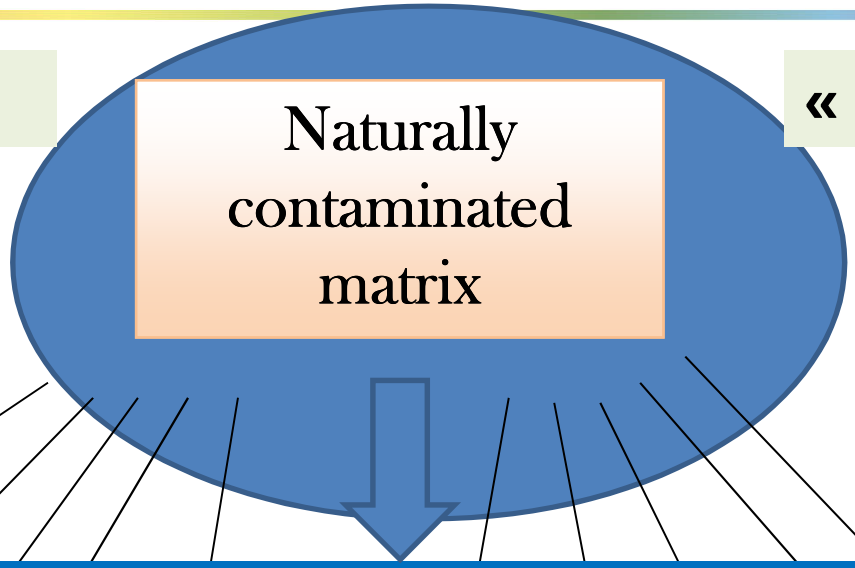
Dilution 1/10 -

Enumeration of *Lm* (EN ISO 11290-2 or validated method)
Return of results to EURL

Trials : test portion« 25g/10g »

« 25g Method »

25 per bag
(5 spots of 5g per bag)



« 10g Method »

10g
(10g of 5 spots per bag)

- **If you have a positive sample stored in your laboratory (>10 ufc.g^{-1})**
- **It could be frozen / fresh**
- **If the sample is > 190g**

Good condition to participate to these trials: to compare a 25g/10g test portion (5 repetitions)

In the near future

You'll receive documents to participate to these trials:

- Survey : who wants to participate to these trials?
- Circular letter
- Protocol
- Results form (by the end of 2015)

lena.barre@anses.fr

Questions

Police | Paragraphe | Style

Lm enumerations will be performed once (no repetition of the analysis) by following the standardised method (EN ISO 11290-2 with amendment) or a validated commercial method. Required material and culture media are described in the protocols or used standards.

3 Preparation of naturally contaminated samples

From a same food matrix naturally contaminated in Lm, perform 10 portions (5 portions of 10 g and 5 portions of 25 g) in the following manner:

- Weigh 10 g taken in 5 spots, corresponding to one test portion. Repeat this operation 5 times, as to get 5 test portions of 10 g each.
- Weigh 25 g taken in 5 spots, corresponding to a test portion. Repeat this operation 5 times, as to get 5 test portions of 25 g each.

4 Samples analyses

In each of the 10 blender bags, perform a 10-fold dilution.
Mix 1 min.
Perform the analyses once. Perfom high dilution rates (until 10^{-6} for instance when the Lm concentration is unknown).

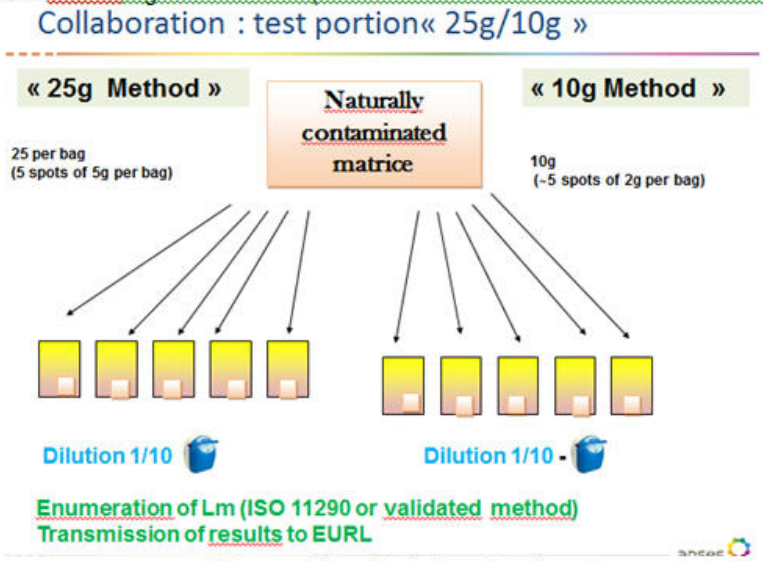


Figure of the detailed protocol

III – TRACABILITY AND RESULTS OF THE PROTOCOL N° 2 (LM ENUMERATION)

RESULT FORM EURL Lm

PROJECT MEASUREMENT UNCERTAINTY / TEST PORTION SIZE ENUMERATION of *L. monocytogenes (Lm)*

- Name & address of the laboratory:
- Description of the sample:
- Details on sample (process: raw, smoked, cooked, marinated, salted, vacuum-packed):
- Context of analysis: self-check, official check (including investigation of listeriosis case):
- If an enumeration of Lm has been previously performed, result obtained (CFU/g):

1. Preparation of the test sample:

Date of analyses:
Method used: ☐ EN ISO 6887 ☐ Other:

2. Enumeration:

Method used for the enumeration: ☐ EN ISO 11290-2 ☐ Other:
Method used for the confirmation (optional):

3. .Results

	Results CFU/ml or CFU/g*
Results of each portion of **10 g**	
Results of each portion of **25 g**	

*: delete where inapplicable

COMMENTS:

(États Unis)

Trials : test portion« 25g/10g »

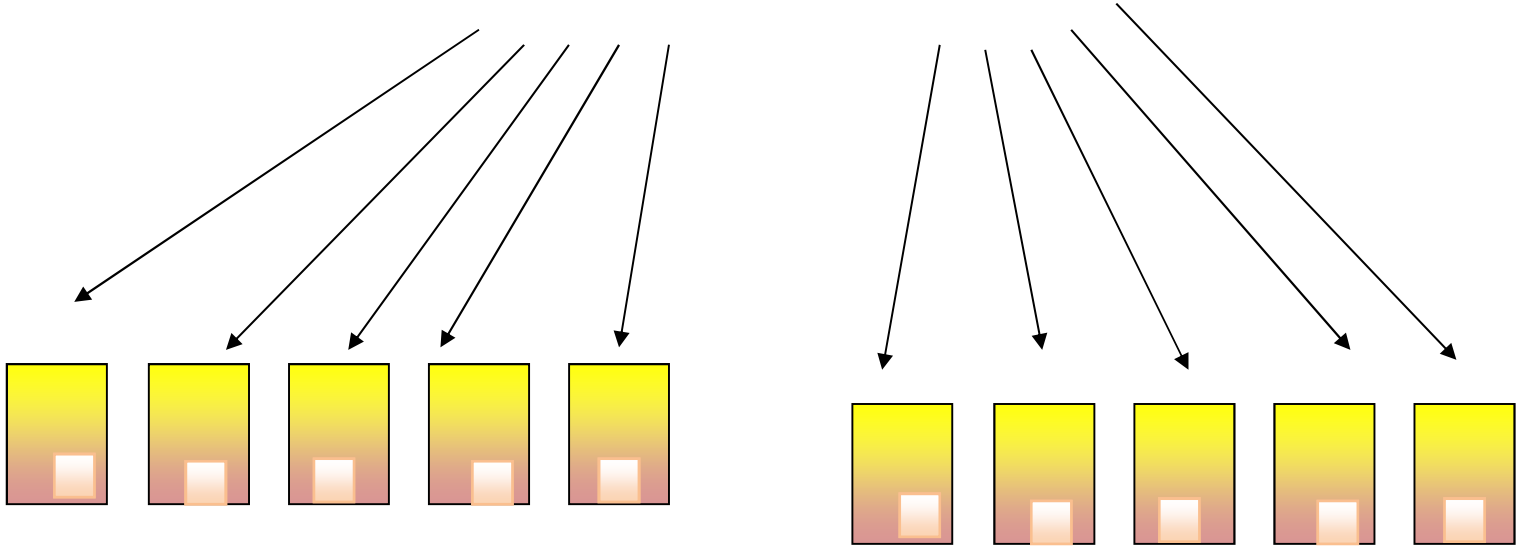
« 25g Method »

« 10g Method »

Naturally contaminated matrice

25 per bag
(5 spots of 5g per bag)

10g per bag
(5 spots of 2g per bag)



Dilution 1/10

Dilution 1/10 -

Enumeration of Lm (ISO 11290 or validated method)
Transmission of résultats to EURL

Conclusion

- **Pour Listeria**

Matrice (nb échantillon)	Résultats (Lm)	Préparation des échantillon (T1) 10g	Préparation des échantillon (T2) 100g
fromage (5)	Moyenne	1,99	1,92
	Ecart-type	0,35	0,13
	Variance	0,13	0,02
Andouille de village (5)	Moyenne	1,76	2,05
	Ecart-type	0,22	0,11
	Variance	0,05	0,01
sauté de porc (5)	Moyenne	1,50	2,64
	Ecart-type	1,35	0,05
	Variance	1,83	0,00
Andouille de guéméné (5)	Moyenne	3,04	4,34
	Ecart-type	1,77	0,01
	Variance	3,15	0,09

Conclusion

- **Pour Flore totale**

Matrice (nb échantillon)	Résultats (flore totale)	Préparation des échantillon s(T1) 10g	Préparation des échantillon s(T2) 100g
fromage (5)	Moyenne	8,59	8,50
	Ecart-type	0,12	0,15
	Variance	0,01	0,02
Poulet basquaise (5)	Moyenne	3,55	3,46
	Ecart-type	0,11	0,05
	Variance	0,01	0,00
Poêlée savoyarde (5)	Moyenne	3,37	4,41
	Ecart-type	0,83	0,07
	Variance	0,69	0,01
pizza royale (5)	Moyenne	3,39	5,26
	Ecart-type	1,36	0,02
	Variance	1,86	0,00
Andouille de village (5)	Moyenne	7,19	7,28
	Ecart-type	0,03	0,04
	Variance	0,00	0,00
sauté de porc (5)	Moyenne	9,44	9,55
	Ecart-type	0,19	0,11
	Variance	0,04	0,01
Andouille de guéméné (5)	Moyenne	8,61	8,12
	Ecart-type	0,38	0,66
	Variance	0,14	0,44

1. Context and objectives
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6. Proposals

Experimental study

Method « 100g »

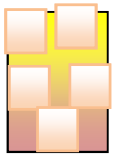
Naturally contaminated matrix

Method « 10g »

100g per bag
(5 spots of 20g per bag)

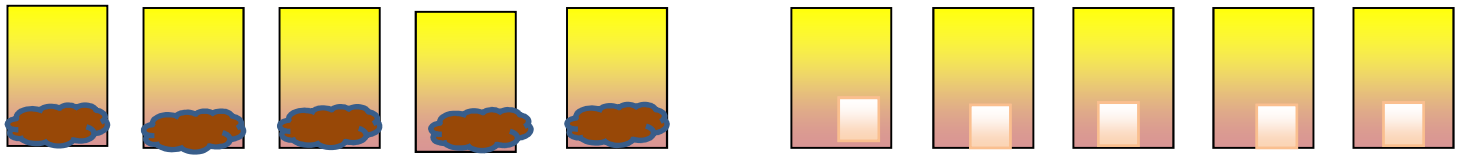
10g (10g in one spot per bag)

1/2 dilution
Stomacher



MU not satisfactory

After the ½ dilution : Difficulty to take the diluent to prepare the 1/5 dilution



20g/bag
+ 80 ml

1/5 dilution - Stomacher

Enumeration *Lm* & total flora

1/10 dilution - Stomacher

Enumeration *Lm* and total flora

Experimental study

Method « 100g »

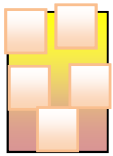
100g per bag
(5 spots of 20g per bag)

Naturally contaminated matrix

Method « 10g »

10g (10g in one spot per bag)

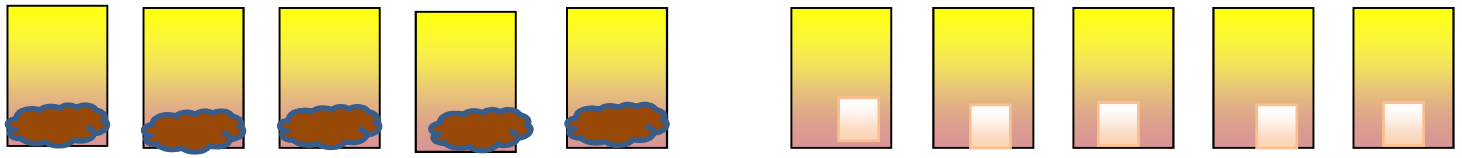
1/2 dilution
Stomacher



MU not satisfactory

After the ½ dilution : Difficulty to take the diluent to prepare the 1/5 dilution

20g/bag
+ 80 ml



1/5 dilution - Stomacher

Enumeration *Lm* & total flora

1/10 dilution - Stomacher

Enumeration *Lm* and total flora

Experimental study : 2 big bags/ 100g

Method « 1/5 and 1/2 »

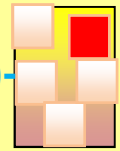
Artificially(?) contaminated matrix

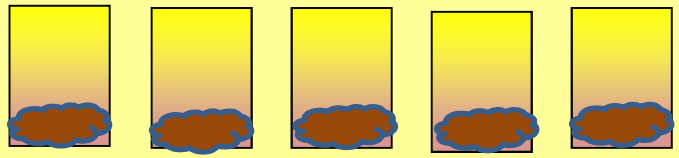
Method « 1/5 » or « 1/3 » or « 1/4 »

100g per bag
(5 spots of 20g per bag
Included ONE spot
contaminated)

Inhibition food cf 9.1 DIS688761

1/5 dilution (400ml)- Stomacher





1/2 dilution (50g+50ml) - Stomacher

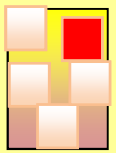
Enumeration *Lm* (& total flora?)

Enumeration *Lm* (& total flora?)

Experimental study: 1big/1small bag/200g

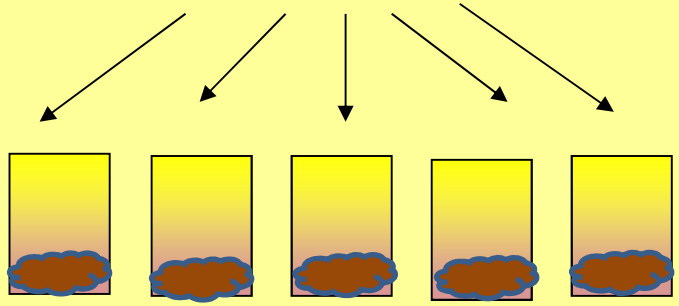
Artificially (?) contaminated matrix

100g per bag
(5 spots of 20g per bag
Included ONE spot
contaminated)



Method «1/3 and 3/10»

1/3 dilution- (200ml) Stomacher



3/10 dilution (30g+70ml) - Stomacher

Enumeration *Lm* (& total flora?)

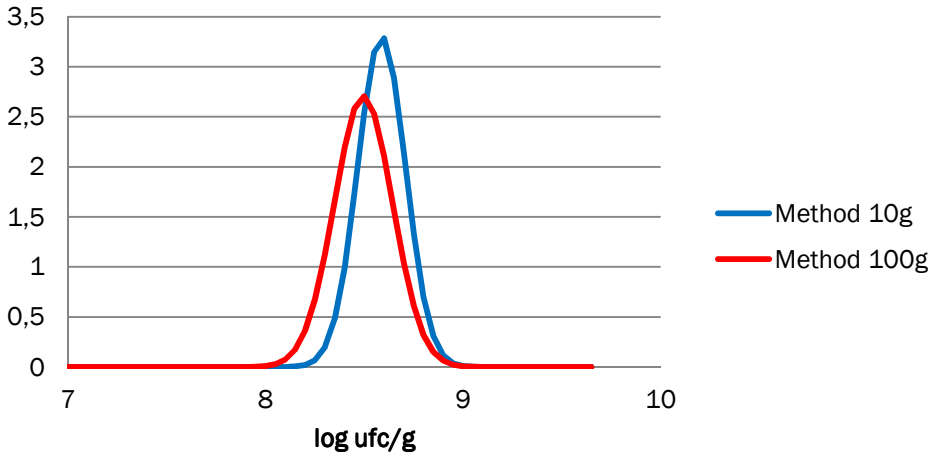
Method « 1/10 »

1/10 dilution - Stomacher

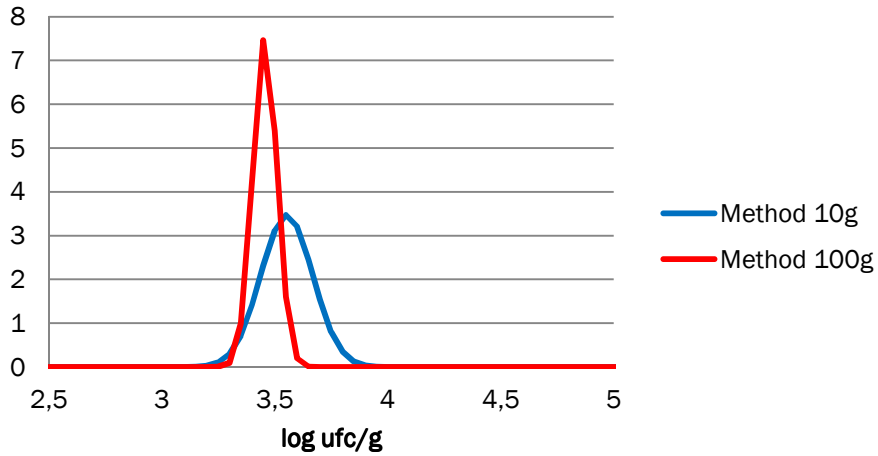
Enumeration *Lm* (& total flora?)

Results : total flora

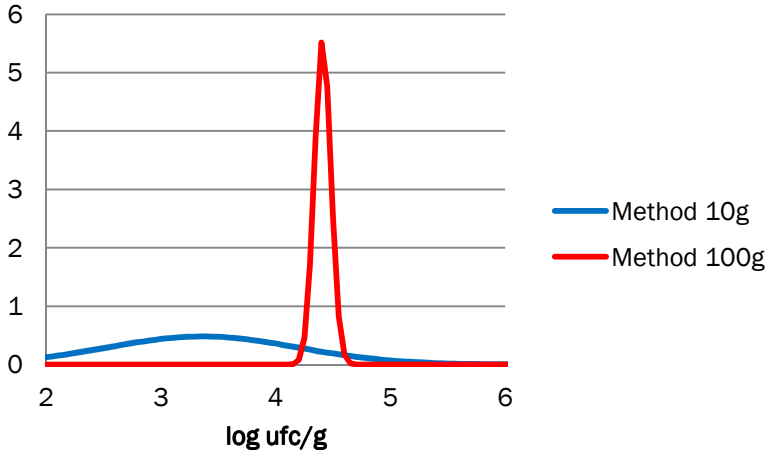
Sample 1 : 10hmpa471.2



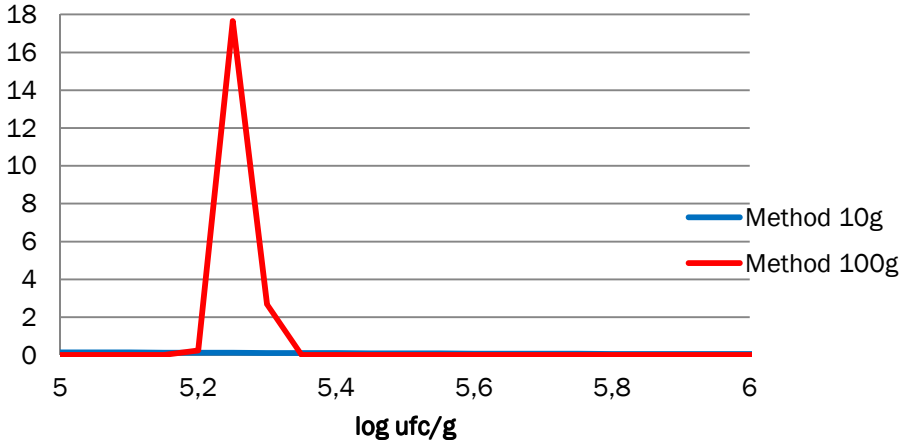
Sample 2 : 12edb947.1



Sample 3 : 12edb949.1

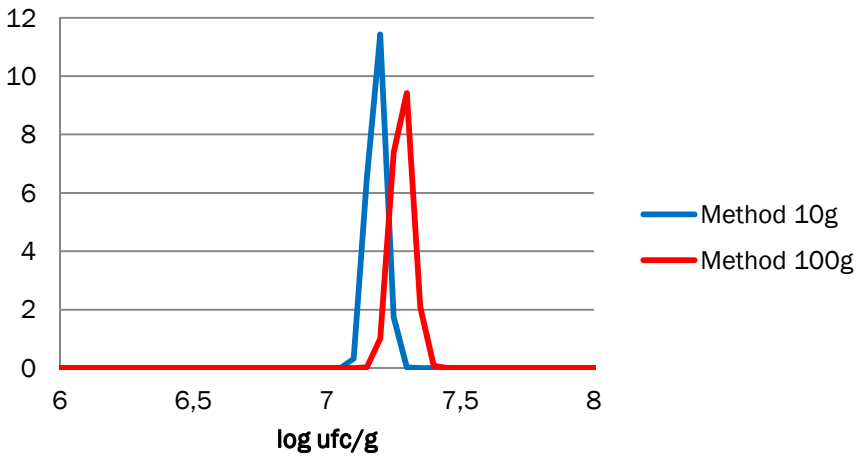


Sample 4 : 12edb931.3

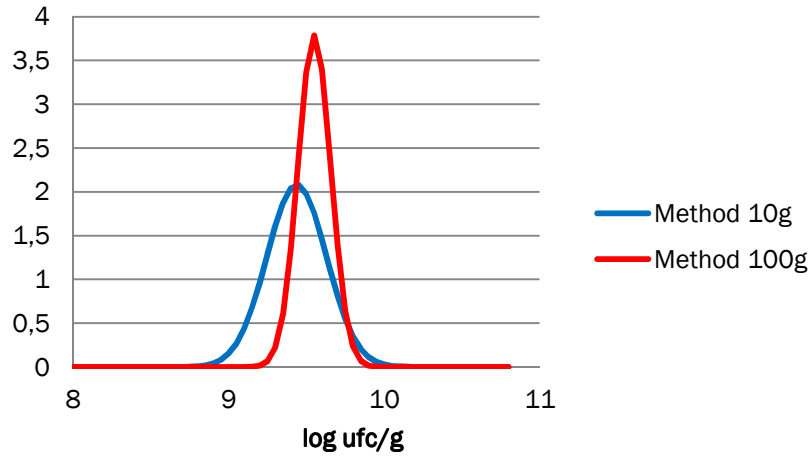


Results : total flora

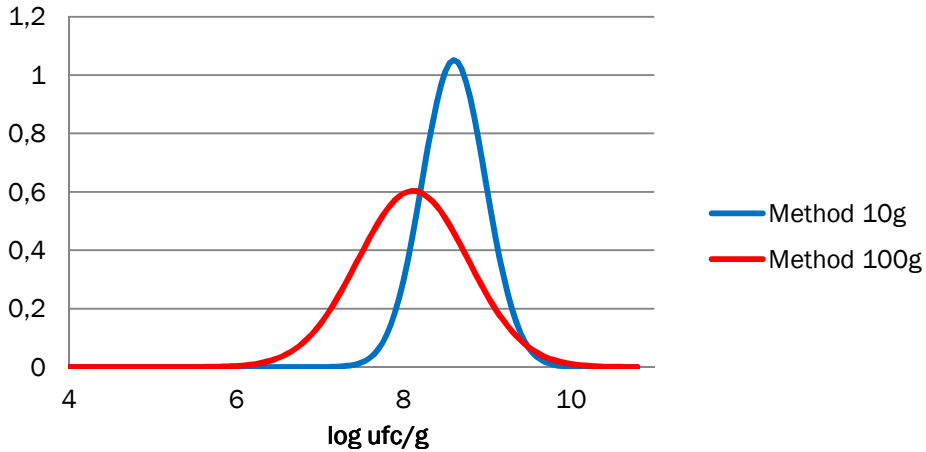
Sample 5 : 12edb1016



Sample 6 : 11edb713

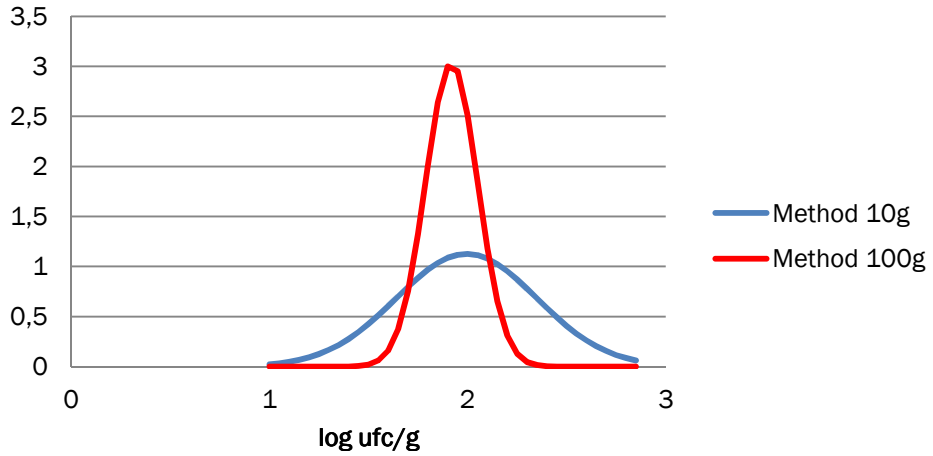


Sample 7 : 10hmpa536

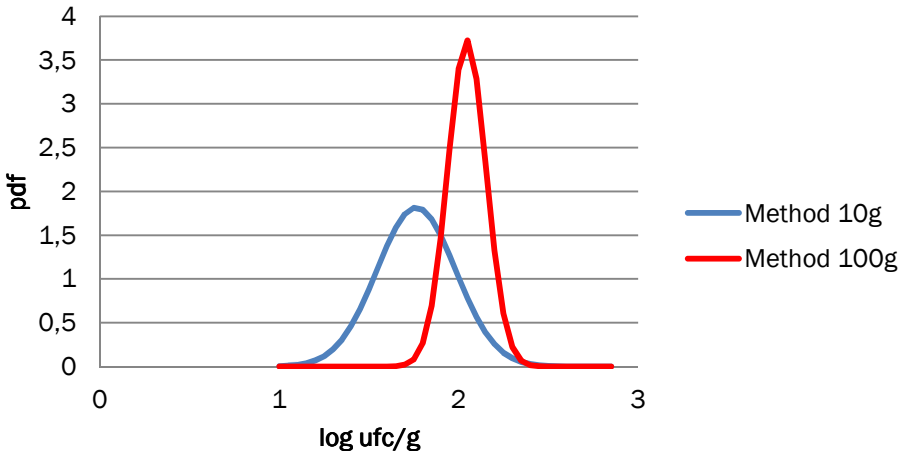


Results: *Listeria monocytogenes*

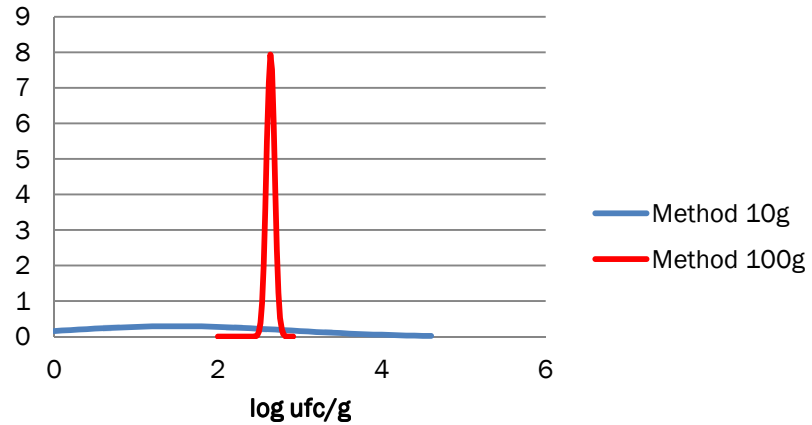
Sample 1 : 10hmpa471.2



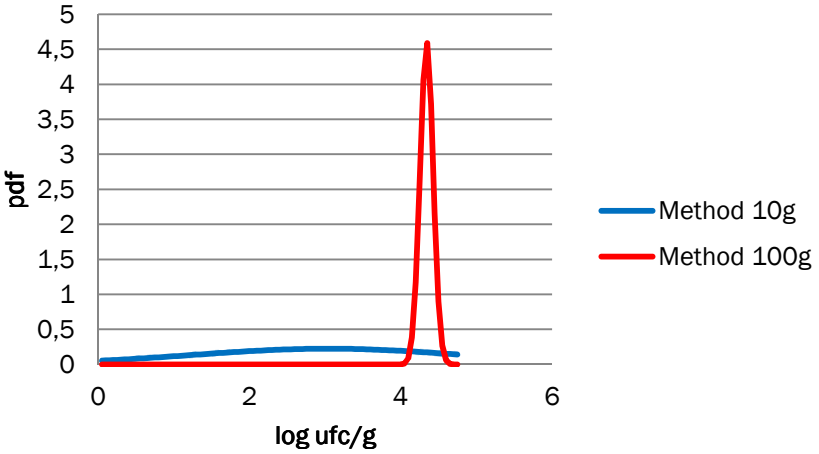
Sample 2 : 12edb1016



Sample 3 : 11edb713



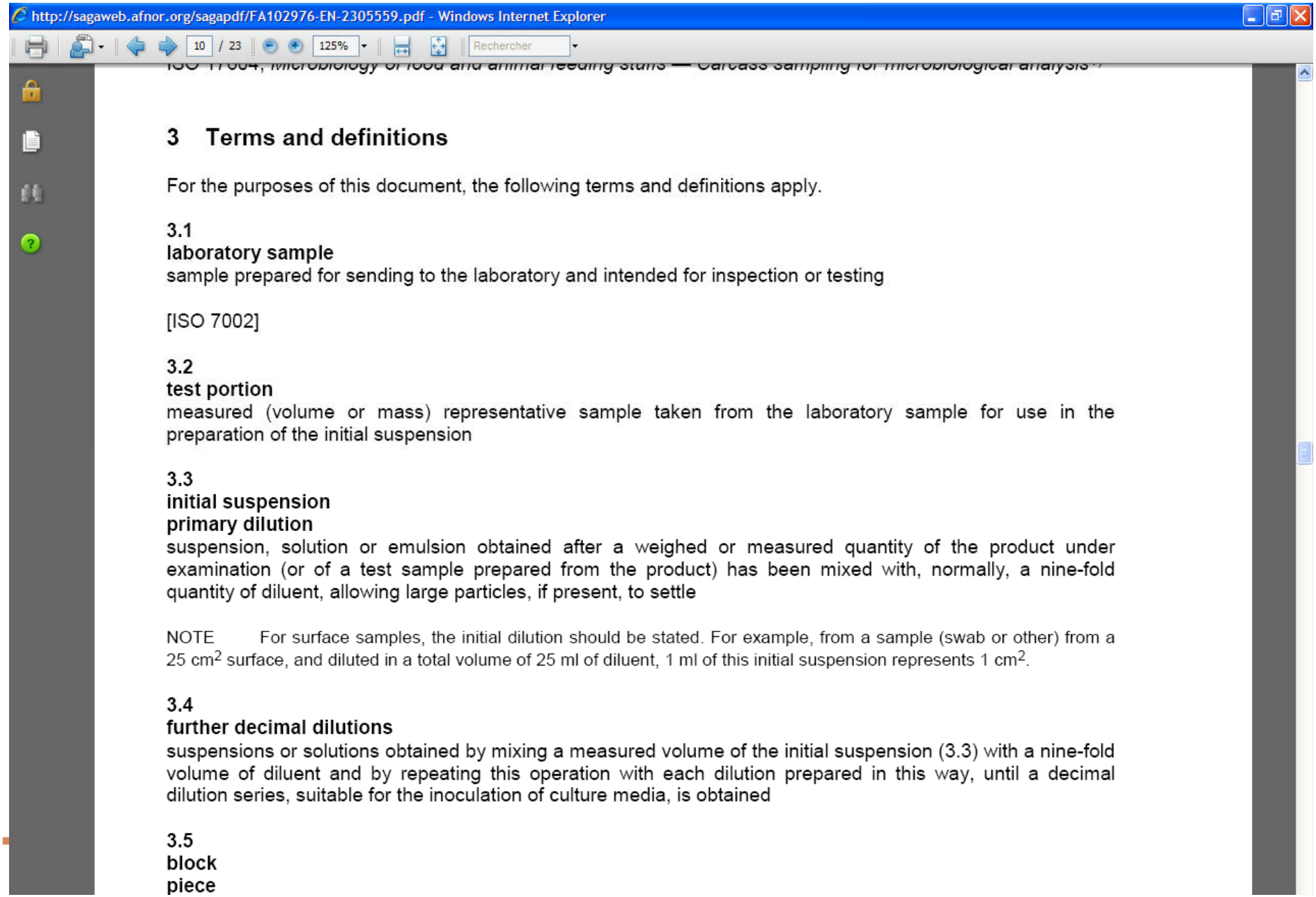
Sample 4 : 10hmpa536



Results: *Listeria monocytogenes*

moyenne A	variance A	ecart type A			moyenne B	variance B	ecart type B
1,995	0,125	0,354			1,919	0,017	0,132
1,764	0,048	0,220			2,046	0,011	0,107
1,499	1,830	1,353			2,643	0,003	0,050
3,038	3,149	1,775			4,343	0,008	0,087

Definitions



ISO 17604, *Microbiology of food and animal feeding stuffs — Carcass sampling for microbiological analysis*[1]

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
laboratory sample
sample prepared for sending to the laboratory and intended for inspection or testing

[ISO 7002]

3.2
test portion
measured (volume or mass) representative sample taken from the laboratory sample for use in the preparation of the initial suspension

3.3
initial suspension
primary dilution
suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed with, normally, a nine-fold quantity of diluent, allowing large particles, if present, to settle

NOTE For surface samples, the initial dilution should be stated. For example, from a sample (swab or other) from a 25 cm^2 surface, and diluted in a total volume of 25 ml of diluent, 1 ml of this initial suspension represents 1 cm^2.

3.4
further decimal dilutions
suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.3) with a nine-fold volume of diluent and by repeating this operation with each dilution prepared in this way, until a decimal dilution series, suitable for the inoculation of culture media, is obtained

3.5
block
piece

Definitions

For hard or dry products, do not homogenize in a rotary homogenizer for more than 2,5 min at a time.

For dry and hard or heterogeneous products, it may be necessary to mince or to grind the laboratory sample. In this case, to avoid an excessive rise in temperature, do not mince or grind for more than 1 min.

7.3 Liquid and non-viscous products

Before analysing, the test sample should be taken after having shaken by hand (e.g. 25 times through an arc of 25 cm; see ISO 8261) or by mechanical means in order to ensure that the microorganisms are uniformly distributed.

7.4 Heterogeneous products

For heterogeneous products (which contain pieces of different foods), sampling should be carried out by taking aliquots of each component representative of their proportions in the initial product.

It is also possible to homogenize the whole laboratory sample to allow the sampling of an homogenized test sample.

It may be necessary to mince or to grind the laboratory sample. In this case, to avoid an excessive rise in temperature, do not mince or grind for more than 1 min.

8 General procedures

Definitions

“It is axiomatic that the cost of analysis increases as the number of sample units to be examined increases, but a cost-benefit relationship exists between this cost and the potential costs of making the wrong decision” (Ramsay et al., 2001).

Bibliography : EN ISO 6887

EN ISO 6887 : preparation of test samples, initial suspension and decimal dilutions for microbiological examination, Part 1 to 5.

EN ISO 6887-6 : Microbiologie des aliments — Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique — Partie 6 : Règles spécifiques pour la préparation des échantillons prélevés au stade de production primaire

E : E : Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 6: Specific rules for the preparation of samples taken at the primary production stage, May 2013

Bibliography : EN ISO 6887

EN ISO 6887-6 :

7 - Types d'échantillons pouvant être envoyés au laboratoire

échantillons prélevés à l'élevage;
dans l'environnement (par exemple chiffonnettes, déchets, matières fécales, poussières, eau);
sur les animaux (par exemple écouvillons);
des échantillons prélevés à l'abattoir (par exemple contenu rectal ou cæcal, ganglions lymphatiques mésentériques);
des échantillons prélevés au couvoir (par exemple fonds de casiers d'éclosoir, coquilles d'œuf cassées);
des échantillons prélevés sur des véhicules, des modules ou des caisses pour le transport d'animaux (par exemple chiffonnettes).

Bibliography : EN ISO 19036

XP ISO/TS 19036
aout 2006
Microbiologie des aliments, Lignes directrices pour l'estimation de l'incertitude de mesure pour les déterminations quantitatives
E : E : Microbiology of food and animal feeding stuffs — Guidelines for the estimation of measurement uncertainty for quantitative determinations

XP ISO/TS 19036/A1
février 2009
Microbiologie des aliments
Lignes directrices pour l'estimation de l'incertitude de mesure pour les déterminations quantitatives
AMENDEMENT 1: Incertitude de mesure sur les faibles taux
E : E : Microbiology of food and animal feeding stuffs — Guidelines for the estimation of measurement uncertainty for quantitative determinations — AMENDMENT 1: Measurement uncertainty for low counts

Context

For ex, EN ISO 6887-1 Standard :

- Homogenize the whole lab sample to allow the sampling of an homogenized test sample
  - Mince or grind the lab sample before adding diluent
  - ½ dilution + 1/5 dilution OR directly 1/10 dilution

Tests on naturally contaminated samples with *Lm*