DEVELOPMENT OF A GELIFIED MATRIX, SUPPORT FOR PROFICIENCY TESTING SCHEMES IN FOOD MICROBIOLOGY

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Raema

réseau d'analyses et d'échanges en microbiologie des aliments

- Creation of **RAEMA** (Réseau d'Analyses et d'Echanges en Microbiologie des Aliments) in **1988**

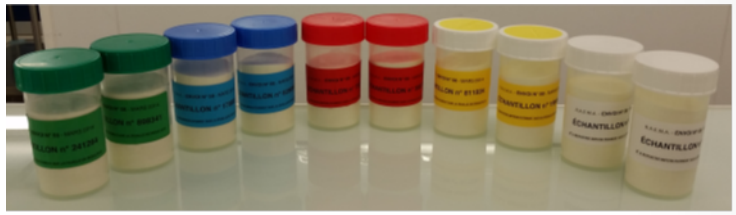
- **RAEMA is our Proficiency Testing Scheme**

- **2007**: **accreditation** of ASA by **Cofrac** , according to **ISO 17043**, as a **Proficiency Testing Scheme Provider**

Raema

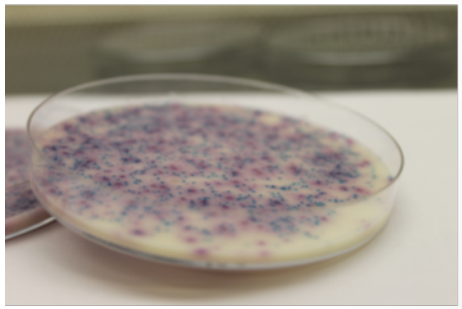
réseau d'analyses et d'échanges en microbiologie des aliments

- **Powder matrix artificially contaminated**



- **Enumerations proposed**:

  Aerobic microorganisms at 30°C
  Enterobacteriaceae
  Total and thermotolerant coliforms
  Beta-glucuronidase-positive *Escherichia coli*
  Coagulase positive *Staphylococci*
  Anaerobic sulfito-reducing bacteria
  Clostridium perfringens
  Listeria monocytogenes



- **Detections proposed**:

  Listeria monocytogenes
  Salmonella

- **Participation** of approximately **400 laboratories**

(France, Belgium, Tunisia, Marocco, Seychelles, …)

To get closer to **samples regularly analysed** by laboratories

To offer new ways to **assess** laboratories **performances**

Development of a supplementary gelified matrix

1- Experimental design

- *Bacillus cereus*
- Lactic Acid Bacteria

2- Results and discussion

1. EXPERIMENTAL DESIGN

1.1. Choice of the matrix

Gelified matrix
Agar subsitute produced from a bacterial substrate

<u>Benefits for laboratories</u>:
- Structure is more similar to the samples usually used
- Manipulation is easier than powder matrix

<u>Benefits for provider</u>:
- Easy to produce
- Easy to include liquid suspension
- Easy to be mixed

→ **homogeneity requirement**

<u>Limits</u>:
- High water activity
- Transport at room temperature

→Trigger a growth of bacteria

⇩

Work on additive compounds to reach **stability requirement**

1. EXPERIMENTAL DESIGN

1.1. Preparation of the bacteria

Bacillus cereus

Vegetative cells

- Incubation of a cryobead in Brain Heart Infusion (BHI)
- Dilution

Sporulated cells

- Spreading liquid suspension on Mannitol egg Yolk Polymixin (MYP) plate
- Recovering the layer

Centrifugation

Preparation of the suspension of contamination

Control of the level of contamination on nutrive and selective media

1. EXPERIMENTAL DESIGN

1.1. Preparation of the bacteria

Lactic Acid Bacteria (*Lactobacillus plantarum*)

- Incubation of a cryobead in Man Rogosa and Sharp broth (MRS)

- Dilution

↓

Centrifugation

↓

Preparation of the suspension of contamination

↓

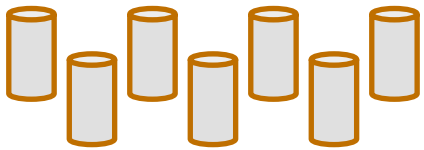
Control of the level of contamination on nutrive and selective media

1. EXPERIMENTAL DESIGN

1.1. Preparation of the gelified matrix

Production of the matrix → **Dispatching in bottles** → **Storage at 25°C and 4°C**

- Permanent stirring
- Sterilization cycle (15 min at 121°C)
- Contamination by bacteria at 45°C



Control of the level of contamination :

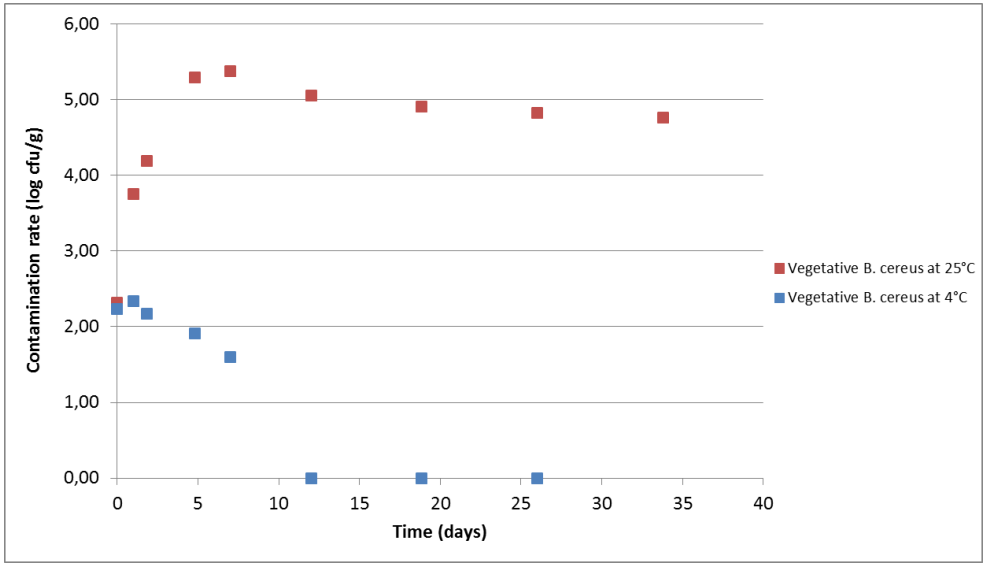
- MYP medium for *Bacillus cereus*
- MRS medium for Lactic Acid Bacteria

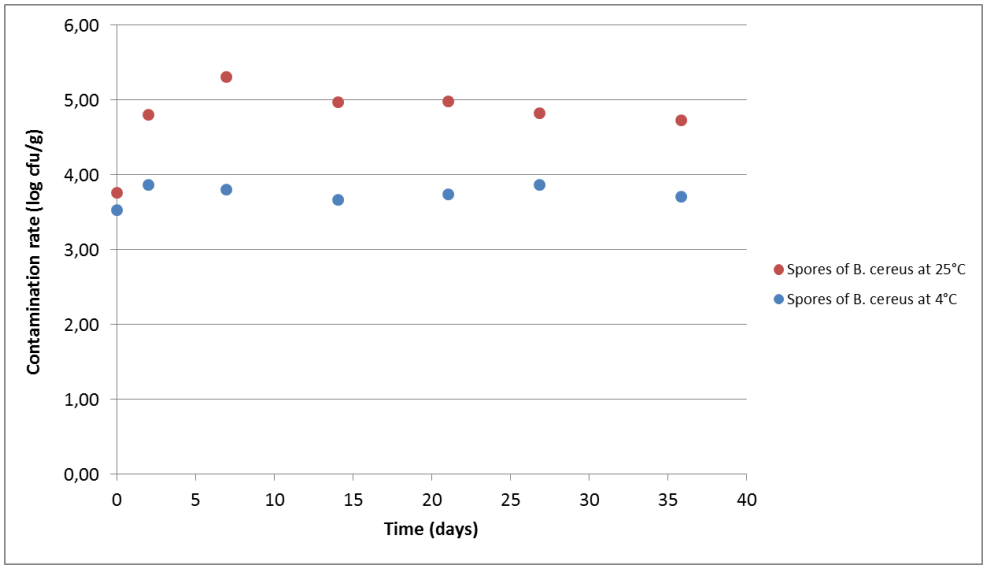
25°C: Transport conditions
4°C: Storage conditions

Control of stability:
3 samples analyzed twice

2. RESULTS AND DISCUSSION

2.1. Stability of *Bacillus cereus*





Vegetative cells of *B. cereus*:

- Growth at 25°C
- Decrease at 4°C
- Lower level of concentration than expected (4 log cfu/g)

Modification of the physiological state of *B. cereus*

Sporulated cells of *B. cereus*:

- Stability at 4°C
- The level of concentration is more similar to the expected one (4 log cfu/g)
- Growth at 25°C

Test bacteriostatic compounds to inhibit growth at 25°C

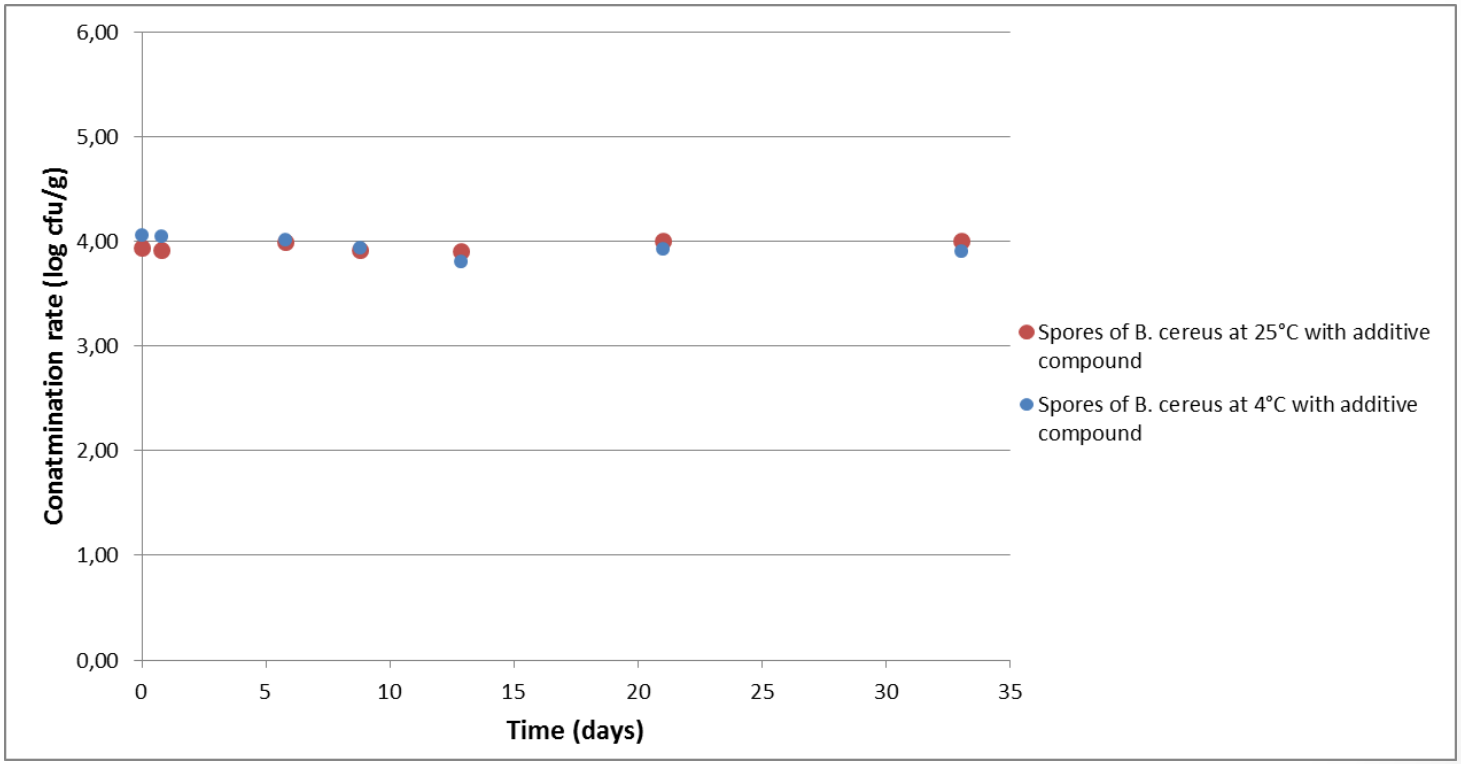
2. RESULTS AND DISCUSSION

2.1. Stability of *Bacillus cereus*

Additional compounds tested: sulfamide, nisin, lactic and acetic acid, essential oils (carvacrol, thymol)…

→ No convincing results obtained

→ Most promising results obtained with one compound



→ Pattern fixed for the preparation of samples submitted in PTS

2. RESULTS AND DISCUSSION

2.2. Stability of Lactic Acid Bacteria

Decrease of LAB over time

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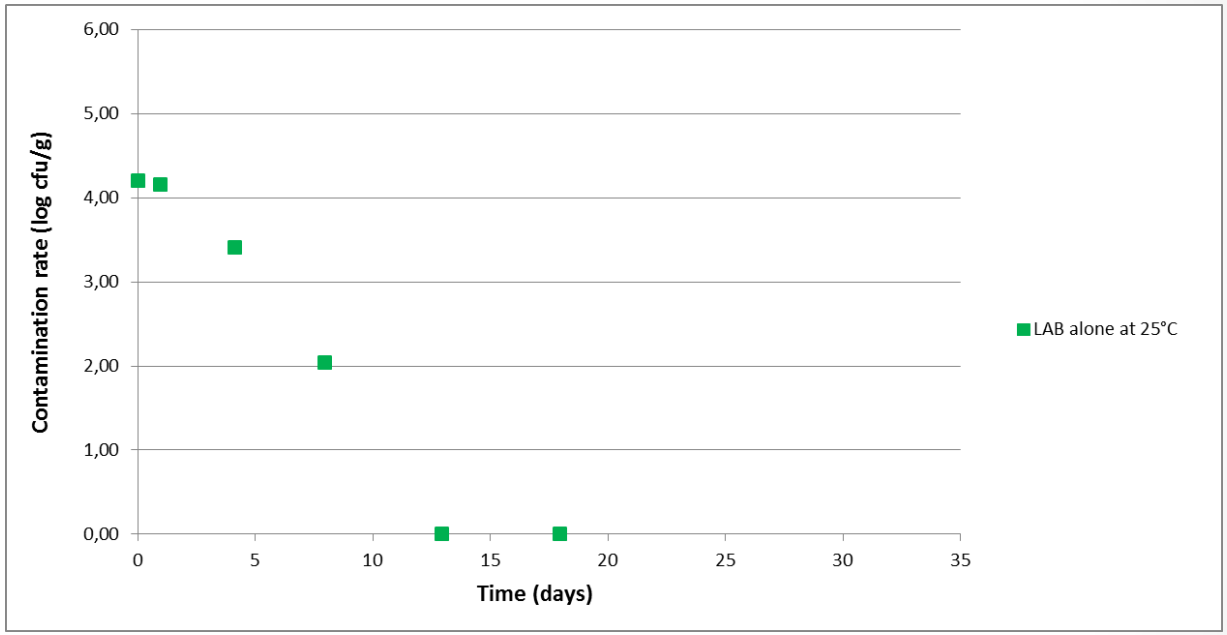
Work to **prevent the reduction of the level of contamination** and to **keep** its **initial level stable**

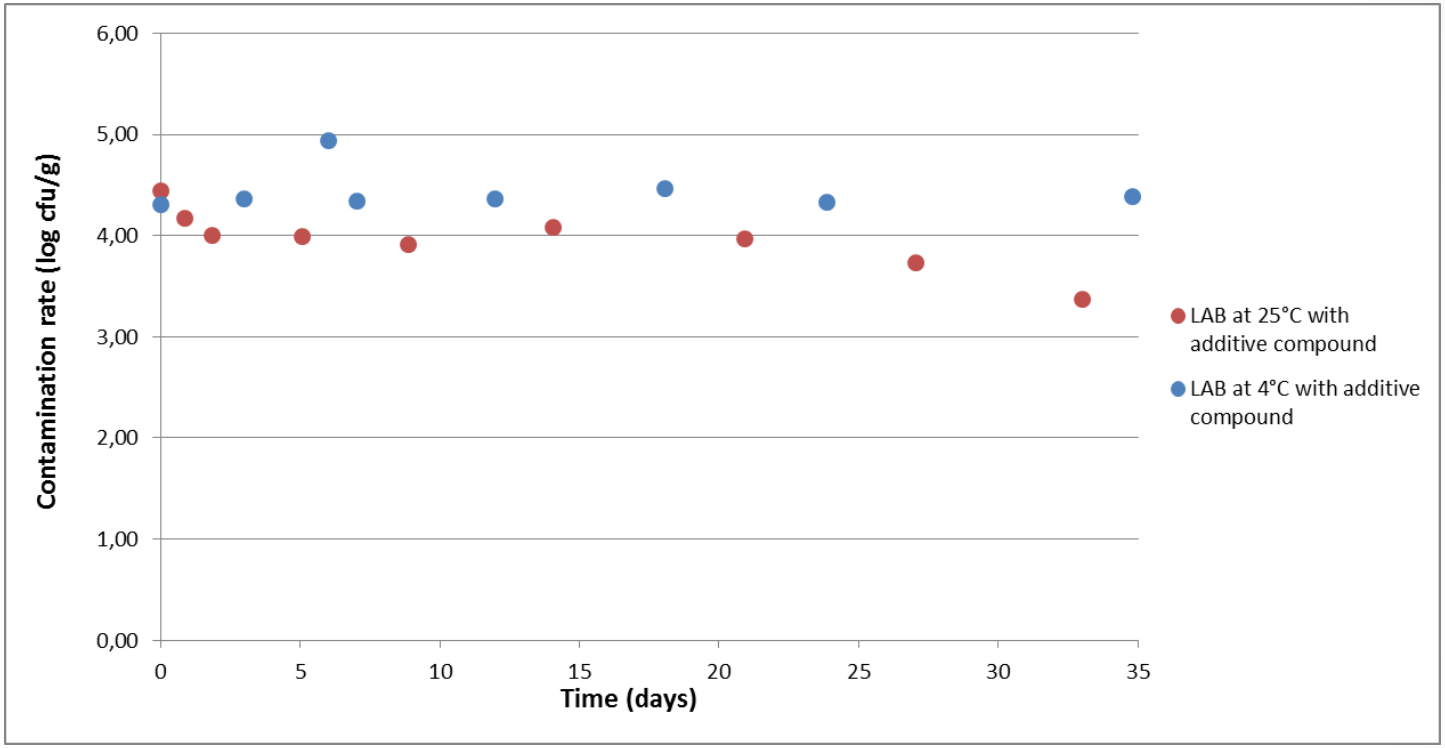


Tested compounds:

- **Protective**: supply a better resistance for bacteria against gelified environment (betain)
- **Nutritive**: supply nutritive elements required for the survival of microorganisms (polyols, sugar, minerals)

2. RESULTS AND DISCUSSION

2.2. Stability of Lactic Acid Bacteria



- The most conclusive result was obtained with a nutritive compound
- Pattern fixed for the preparation of samples submitted in PTS

2. RESULTS AND DISCUSSION

2.3. Homogeneity of contamination level of bacteria

Calculation of 2 parameters to control **homogeneity** (10 samples in duplicate):

- **Homogeneity between samples** : variance of the 10 means
- **Homogeneity within samples** : mean of the 10 variances

	Variance within samples (log cfu/g)	Variance between samples (log cfu/g)
Bacillus cereus	0.018	0.030
Lactic Acid Bacteria	0.003	0.006

➡ Values comparable to values obtained on powder matrix

➡ Results made it possible to set up the Proficiency Testing Scheme

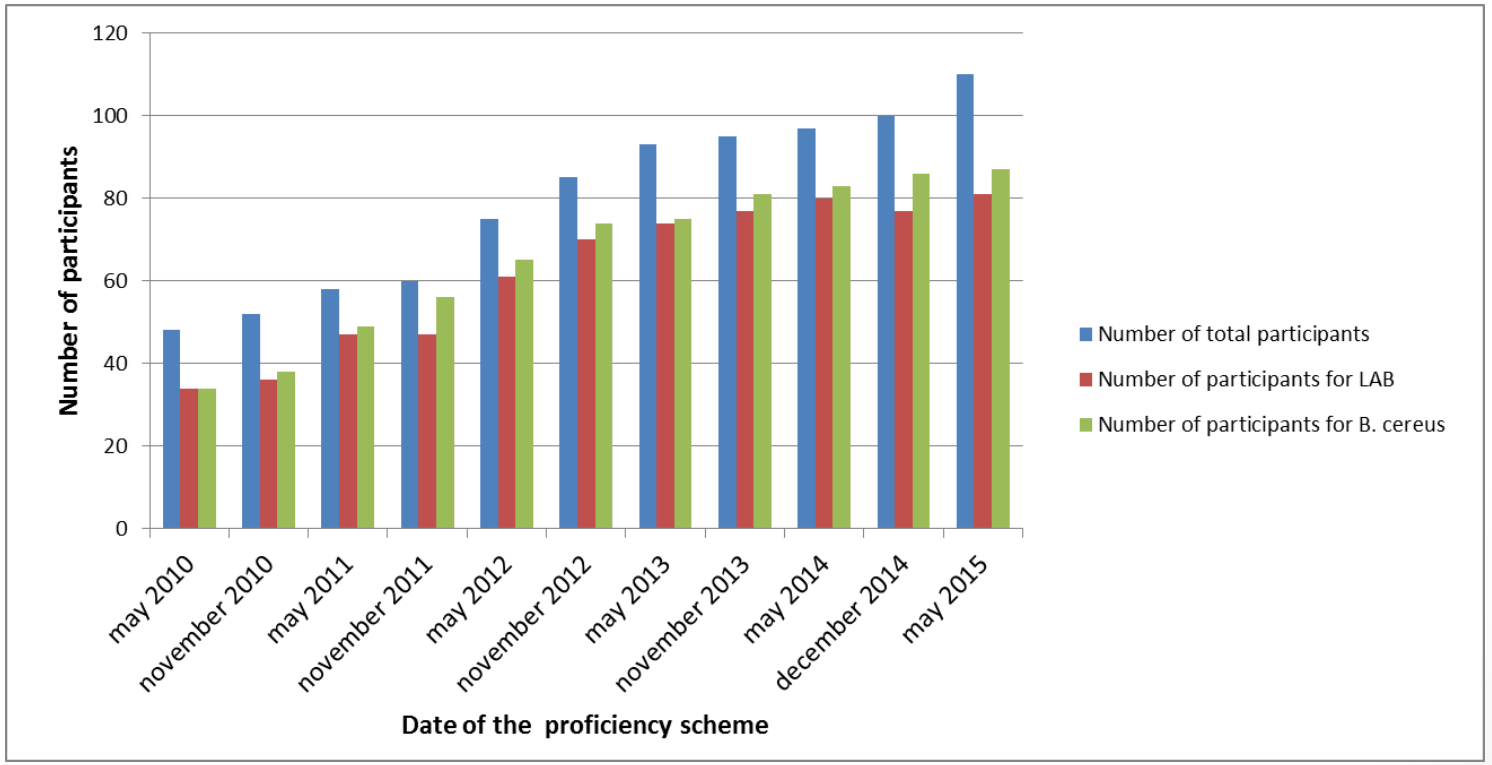
2. RESULTS AND DISCUSSION

2.4. Proficiency testing scheme

2010: creation of 2 Proficiency Testing schemes per year: **supplementary RAEMA**

- Contamination of the samples by just one type of bacteria
- Choice is made by laboratories in the menu

Increase of the **number of participants**

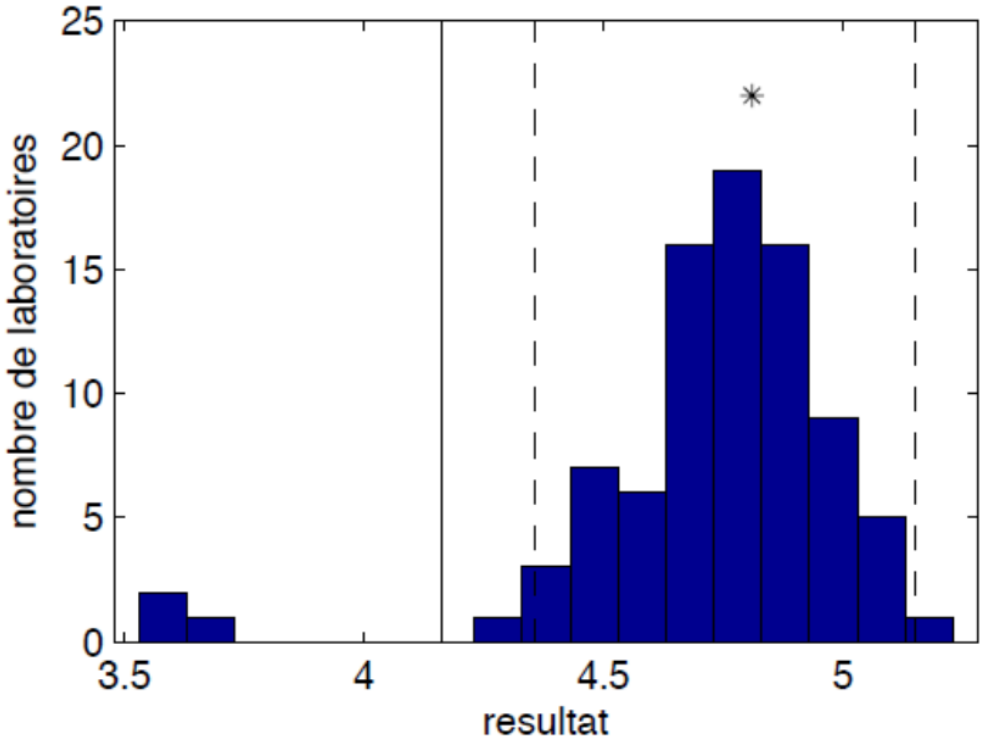


2. RESULTS AND DISCUSSION

2.4. Proficiency testing scheme

- Assessed laboratory performance:
  - **Trueness** evaluation

- Individual report:
  - **z-score**: statistic of individual performance



2. RESULTS AND DISCUSSION

2.4. Proficiency testing scheme

► General report:

- **General data** on analytical techniques used by laboratories
- Factors influencing the results of laboratories

1. EXPLOITATION DES COMPTES RENDUS D'ANALYSES

1.1. TAILLE DE LA PRISE D'ESSAI

108 laboratoires la précisent.
La taille moyenne est de **13.8 g** avec un écart-type de 7.0 g. La taille minimale renseignée est 1 g et la taille maximale 46 g.

1.2. TECHNIQUES D'HOMOGÉNÉISATION UTILISÉES

106 laboratoires homogénéisent leur prélèvement avec un Stomacher[ND]. Trois laboratoires utilisent une technique autre.
La durée moyenne est de **2.2 min** avec un écart-type de 0.9 min. La durée minimale renseignée est 1 min et la durée maximale 3 min.

1.3. CONDITIONS DE REVIVIFICATION

1.3.1. DURÉE

99 laboratoires la précisent.
La durée moyenne est de **22.1 min** avec un écart-type de 11.7 min. La durée minimale renseignée est 1 min et la durée maximale 60 min.

1.3.2. TEMPERATURE

99 laboratoires la précisent.
La température moyenne est de **20.9°C** avec un écart-type de 1.7°C. La température minimale renseignée est 19°C et la température maximale 27°C.

1.1. BACILLUS CEREUS

86 laboratoires réalisent le dénombrement.

Méthode	Nb laboratoires
NF EN ISO 7932	58
AES 10/10-07/10	12
BKR 23/06-02/10	11
Autres	5

Method

Milieu	Nb laboratoires
Mossel	53
BACARA	18
Compass	12
Autres	3

Culture medium

Mode de préparation	Nb laboratoires
Sur place	10
Prêt à l'emploi non pré-coulé	10
Prêt à l'emploi pré-coulé	66

Preparation

	Oui	Non
Traitement thermique	1	85

Mode d'ensemencement	Nb laboratoires
En surface	76
Dans la masse	9

Seeding way

Température d'incubation	Nb laboratoires
30°C	85
37°C	1

Durée d'incubation	Nb laboratoires
24-25 h	40
42-48 h	38
18-23 h	8

Incubation temperature and duration

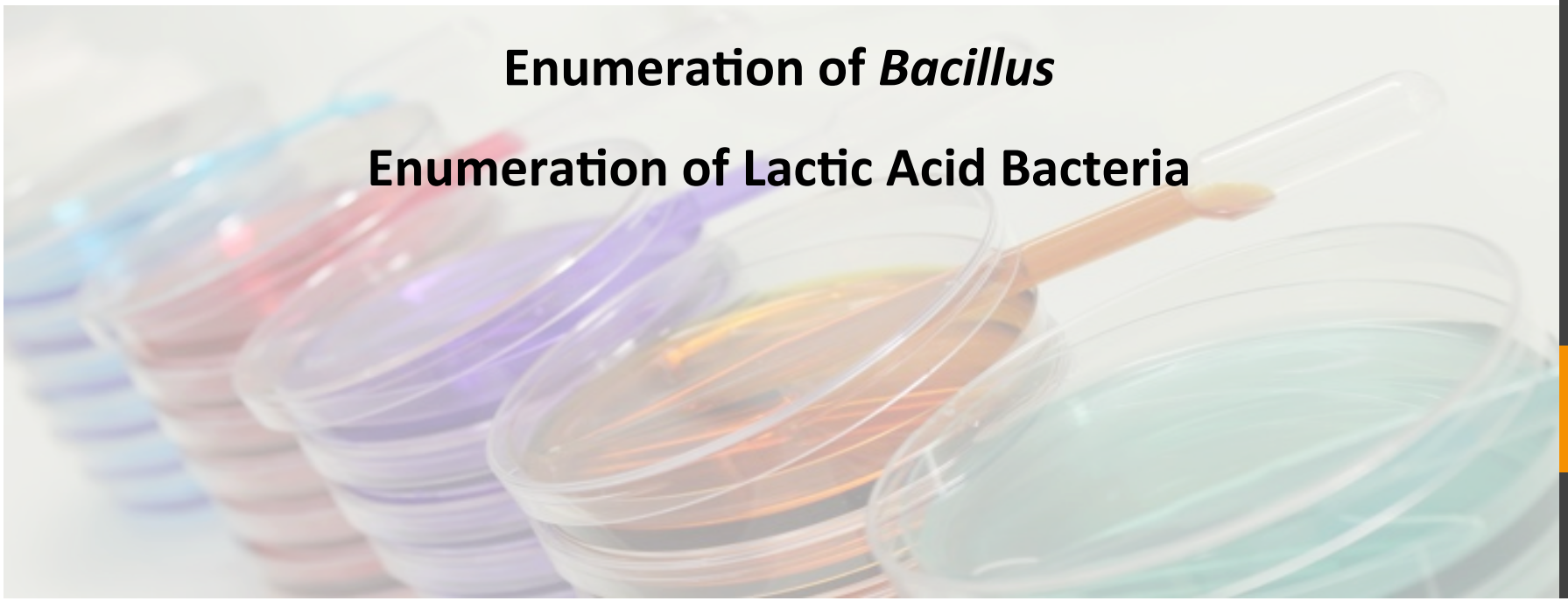
Confirmation	Nb laboratoires
Biochimique (dont hémolyse)	44
Aucune	39
Autres	3

Confirmatory test

2. RESULTS AND DISCUSSION

2.4. Proficiency testing scheme

July 2015: **accreditation** of ASA by **Cofrac**, according to **ISO 17043**, to organize and supply **supplementary RAEMA** for the 2 parameters:



Enumeration of *Bacillus*

Enumeration of Lactic Acid Bacteria

DEVELOPMENT OF A GELIFIED MATRIX, SUPPORT FOR PROFICIENCY TESTING SCHEMES IN FOOD MICROBIOLOGY

- **New ways** to **assess** laboratories **performances**
- Other **parameters** proposed by **supplementary RAEMA**:

- Enumeration of ***Pseudomonas***

- Enumeration of **yeast and moulds**

THANK YOU

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