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Laboratory for Food Safety
Maisons-Alfort location

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes
http://eurl-listeria.anses.fr

Comparison of inoculation techniques of solid food matrices

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Objectives of the study

- **Purposes:**

⇨ To improve the method currently used by EURL *Lm:* method/matrix

⇨ To simulate a naturally occurred contamination (structure, physico-chemical parameter, microflora…)

⇨ To take into account the subsampling stage in the PT trials

⇨ To provide new inoculation techniques for NRLs which organize PT trials at national level

⇨ To investigate artificial contamination of very low numbers of cfu/samples

⇨ To test new matrices

To identify the different inoculation techniques used to contaminate solid food matrices and to compare them

- **Parameters to be taken into account:**

⇨ **Adaptation to structure of matrices (slice, cube-shaped…)**

⇨ **Volume, surface, weight of pieces of matrices.**

⇨ **To allow inoculation of a large volume of matrices.**

⇨ **Rapid and easy to set up**

⇨ **Need of a particular know-how?**

⇨ **Safety for manipulators**

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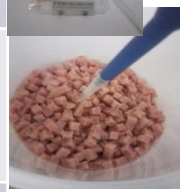
  - ⇨ **Safety for manipulators**

Bibliography study

Technique	Food matrices	Structure of matrice	Material
One by one	Salmon, salad, diced chicken…	cube-shaped, slice	Pipette
Blending contamination	Cheese, Fruit portion, diced of ham, chicken	Pieces of product	Professional Blender
Dry inoculation	Almonds, walnuts and pistachios, butter, Powder	Entire product	(Beads, silicium or CaCo3 powder)
Dipping	Salade, chicken legs	Entire product,	Cuve, tray
Spraying	Mortadella,	slice	Aerograph
Spreading	Salmon	slice	spreader


Experimental study

Two methods compared:

- ⇨ One by one (currently used in the lab)
- ⇨ Blending contamination

Matrix : cube-shaped matrices (diced poultry)

- Pilot study: (300g contaminated ⇨ sampling 20x10g)
  - Single analysis ⇨ Single analysis (whole sample analyzed)
  - Duplicate analysis⇨ subsampling of sample

- Real scale study: (1,5kg contaminated ⇨ sampling 60x20g)
  - Duplicate analysis⇨ subsampling of sample
  - Technical comparison

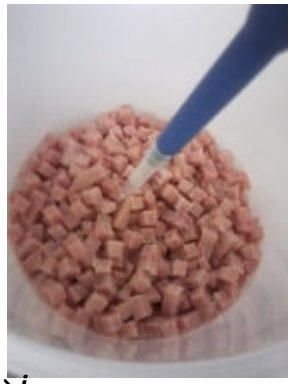
Experimental design: single analysis

One by one

Blending contamination

Contamination
≈5 log (cfu.g$_{-1}$)
1% v/w

Blending

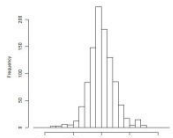


sampling stage

i_1 i_2 i_3 i_4 i_5 $i_x \rightarrow i_{20}$

Enumeration
ALOA or PALCAM

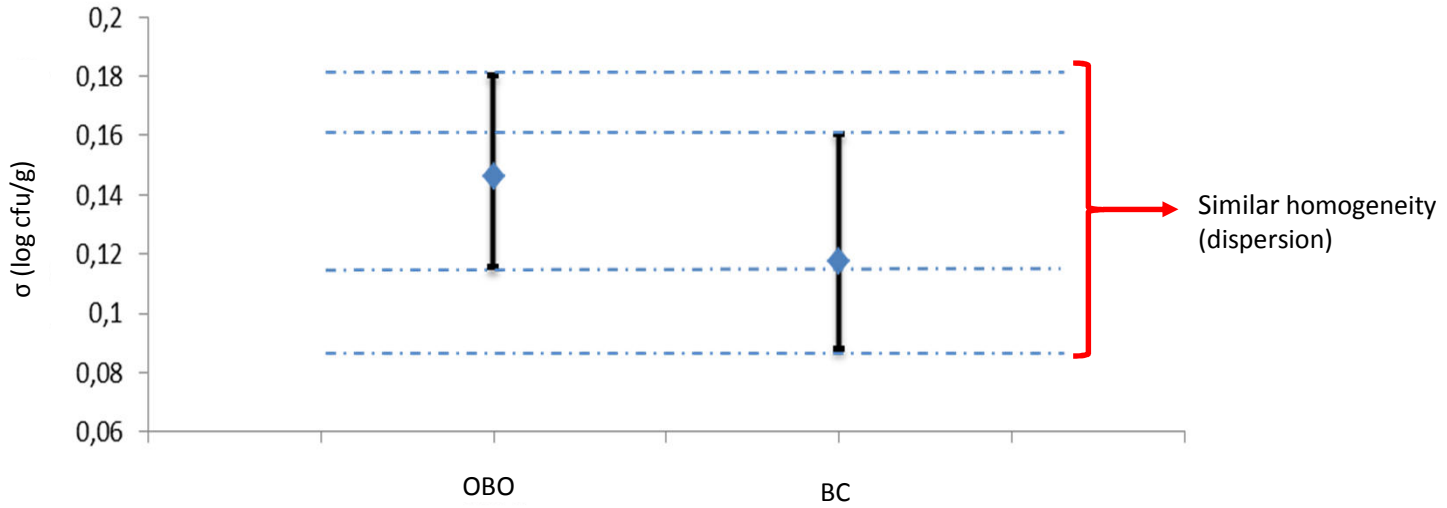
μ, σ i_1 i_2 i_3 i_4 i_5 $i_x \rightarrow i_{20}$



Statistical analysis

i_1 i_2 i_3 i_4 i_5 $i_x \rightarrow i_{20}$ μ, σ

Single analysis: whole sample



Credibility interval (95%) comparison: Data collection of 3 batches centralized to 0, (Nbootstrap= 1000; Fitted N(μ;σ)

- Similar homogeneity
- Less reproducible with OBO technique.
- Contamination targeted is most difficult to reach with OBO

Experimental design: Duplicate analysis

One by one

Contamination
≈5 log (cfu.g$_{-1}$)
1% v/w

sampling stage
)

$i_x \rightarrow i_{10}$ …

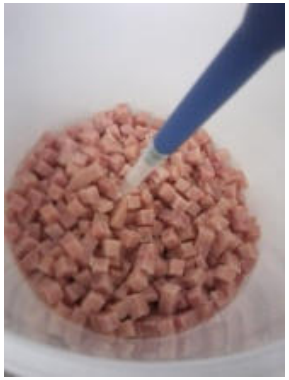
i_1 i_2 i_3

i_{1a} i_{1b} i_{2a} i_{2b} i_{3a} i_{3b}

$i_{xa}/i_{xb} \rightarrow i_{10}$ …

Blending contamination

Blending



$i_x \rightarrow i_{10}$ …

Analyze in duplicate
Subsampling stage
(PT trial participant)

i_1 i_2 i_3

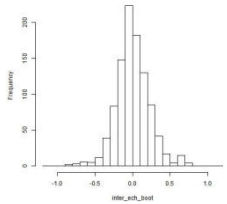
i_{1a} i_{1b} i_{2a} i_{2b} i_{3a} i_{3b}

$i_{xa}/i_{xb} \rightarrow i_{10}$ …

Enumeration
ALOA or PALCAM

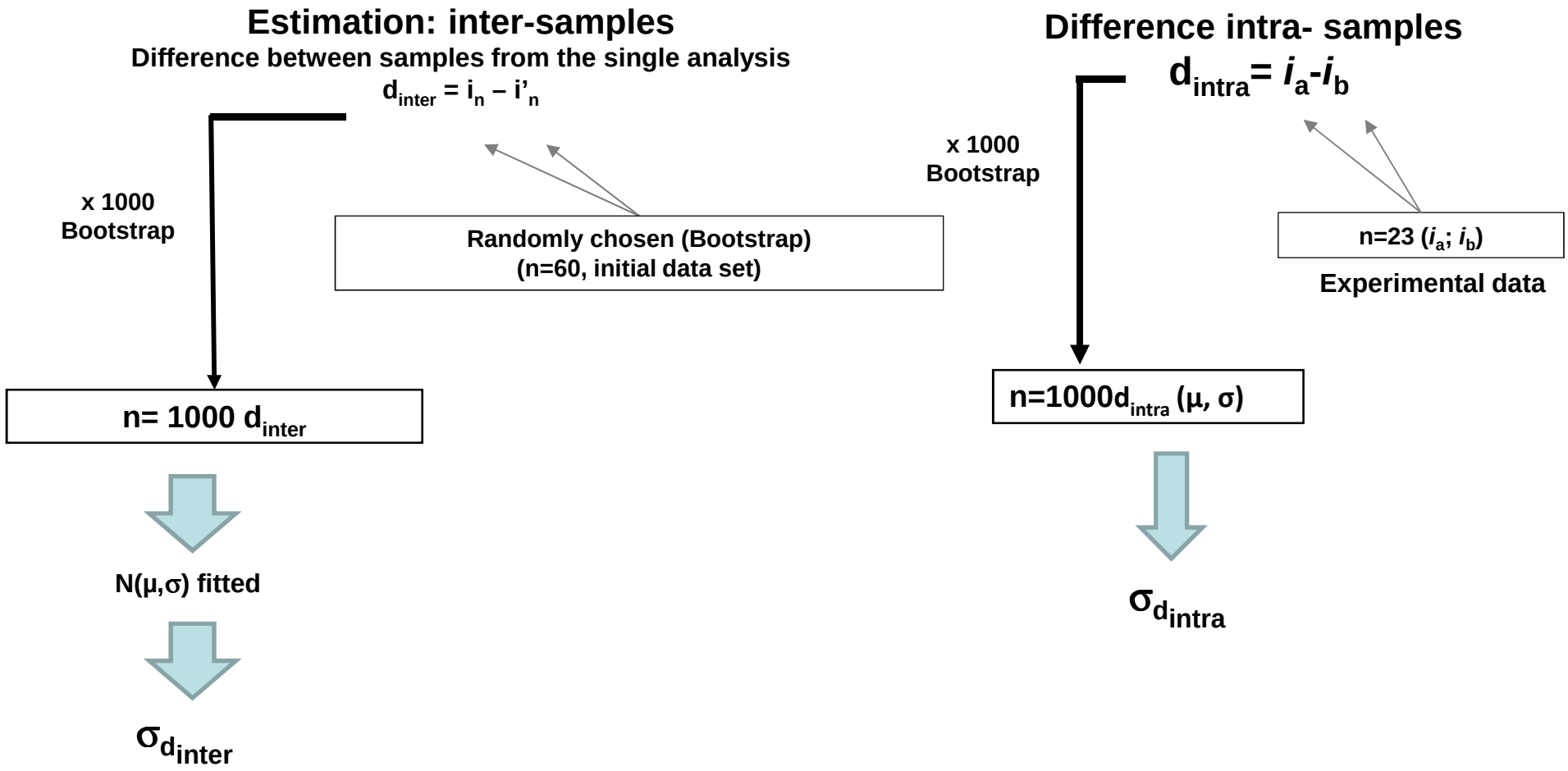
BC

μ, σ { $i_{1(a;b)}$ $i_{2(a;b)}$ $i_{3(a;b)}$ $i_{xa;b)} \rightarrow i_{10}$ } μ, σ

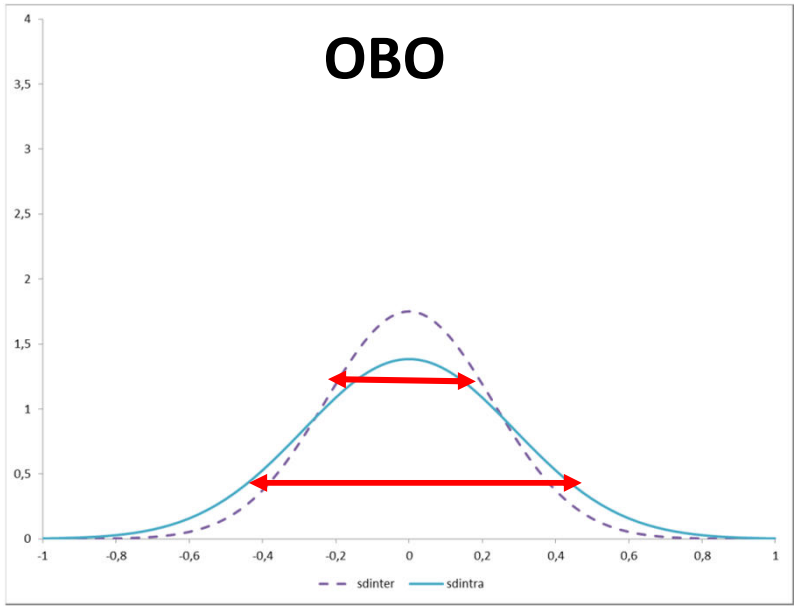


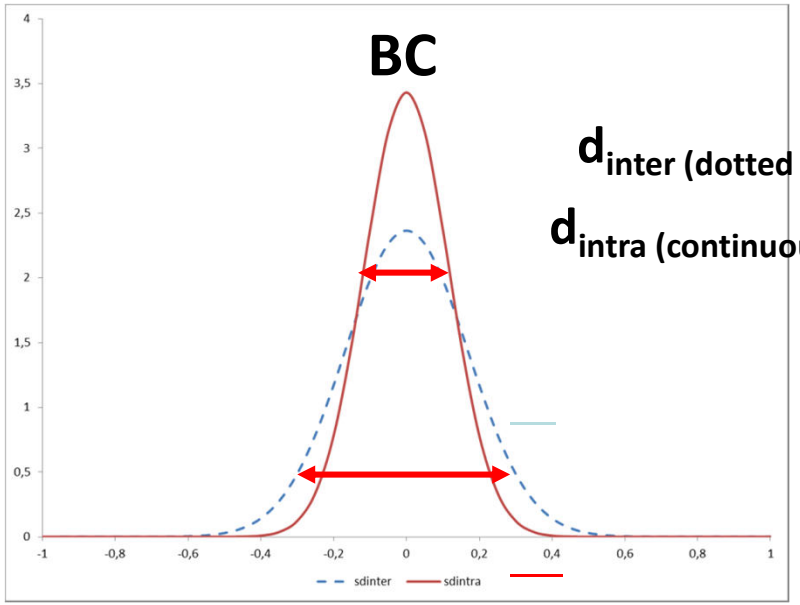
Statistical analysis

Inter-samples and intra-samples differences comparison



Comparison of d_{intra} vs d_{inter} and d_{intra} OBO vs d_{intra} BC





d_{inter} (dotted line)

d_{intra} (continuous line)

Tech	σ	σ_d	$\sigma^2_{dintra}/\sigma^2_{dinter}$
OBO	σ_{dinter}	0,22	1,60
	σ_{dintra}	0,28	
BC	σ_{dinter}	0,17	0,50
	σ_{dintra}	0,11	

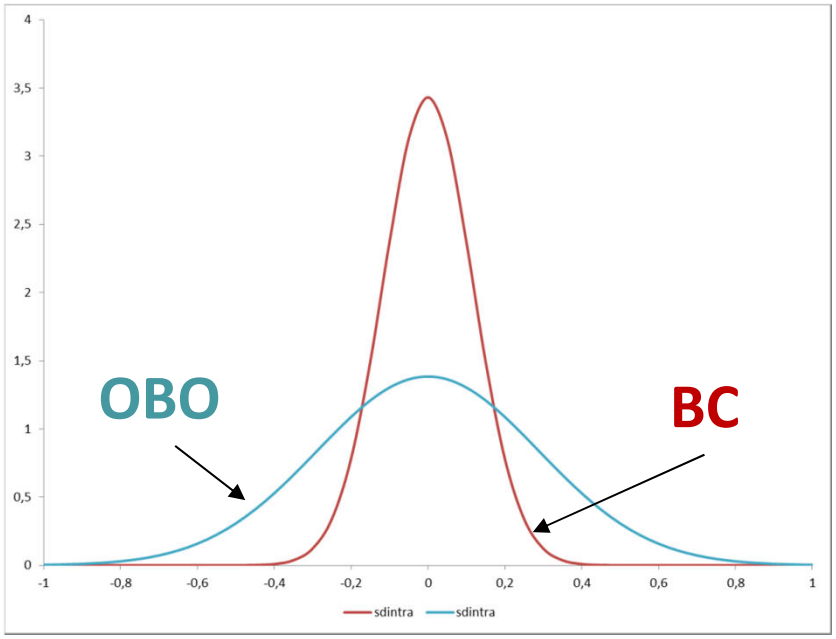
Subsampling

Increased

Heterogeneity

Decreased

Comparison of d_{intra} OBO vs d_{intra} BC



Tech	$\sigma_{d_{intra}}$	$\sigma^2_{d_{intra}}/\sigma^2_{d_{intra}}$	subsampling
OBO	0,28	6,14	Not Adapted
BC	0,12		Adapted

Blending contamination: Interlaboratory scale

Food matrix: 1,5kg (Diced poultry)

- 60 samples (25g) ⇨ 2 x10g (subsampling)
- 10 samples: homogeneity study
- 6 samples: stability study
- 44 samples : participant's network

Range of contamination level :

- 150 to 10000 cfu/g and 5 cfu
- 5 cfu/g

Contamination:

- Inoculum (L. monocytogenes suspension at 2 log upper to the target)
- Blending (5 x 2 min)

Homogeneity study:

- 10 samples randomly selected were analyzed in duplicate

Blender (10L)

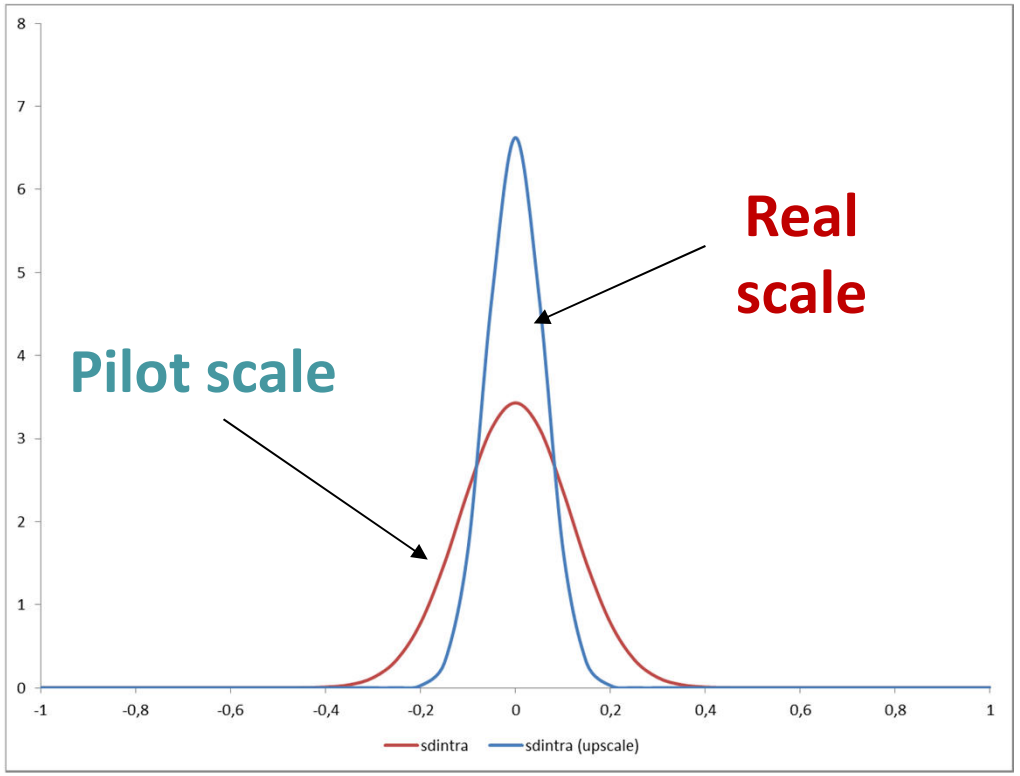
Interlaboratory scale: contamination level targeted 10000 cfu/g

Level of contamination targeted reached at around ±0,05 log

scale	σ_{dintra}	$\sigma^2_{dintraP}/\sigma^2_{dintraR}$
Pilot	0,11	3,72
Real	0,06	

⇨Homogeneity satisfactory

⇨Homogeneity intra-samples satisfactroy



n=40 (2 batch of 10)

Interlaboratory scale: contamination level targeted 150 cfu/g

Enumaration low number (<30 colony per plate)
Homogeneity assessed by the T1-T2 test (based on Poisson distribution recomanded by the EN ISO/TS 22117:2010)

		Intra-sample	Inter-sample
Batch	[*Lm*] log cfu/g	T1 (Intra-sample)	T2 (Inter-sample)
1 (2x10)	2,18 (151)	satisfactory	satisfactory
2 (2x10)	2,12 (132)	satisfactory	satisfactory

⇨Homogeneity intra and inter samples satisfactory

Contamination technique adapted to prepare low contaminated samples

Interlaboratory scale: contamination level targeted 5 cfu/sample

Detection of low number (≈ 5 colony per 25g, 50g sample analyzed in duplicate)

Poisson distribution λ=4x20

2	4	3	3	6
5	5	4	3	3
4	4	6	1	0
4	2	5	2	2

Negatif sample: p=0.02

Poisson distribution λ=5x20

5	7	2	2	5
10	2	2	7	7
5	3	6	3	2
3	5	7	1	6

Negatif sample: p=0.007

Example of distribution not homogenous (≠ Poisson distribution)

0	7	1	2	5
12	2	2	7	7
5	3	0	3	2
3	14	7	1	18

Not expected λ=5x20

Target 5 cfu/25g

Batch	[*Lm*] cfu/sample	Enrichment				Final results Positif/total		Most adapted
		Half Fraser		Fraser				
		OBO	BC	OBO	BC	OBO	BC	
1 (2x10)	5-6	19/20	20/20	19/20	20/20	19/20	20/20	
2 (2x10)	6-7	20/20	20/20	20/20	20/20	20/20	20/20	
3 (2x10)	6-7	20/20	20/20	20/20	20/20	20/20	20/20	

Conclusion

Technical comparison : (Pros/cons)

- ⇨ OBO is adaptated for various type of matrice (slice, dice… etc)
- ⇨ OBO preserved the structure of matrice better than BC
- ⇨ BC is less laborious (less step, time consuming, operator and manipulattion of sample)
- ⇨ More Critical point in OBO (Contamination and homogenisation)
- ⇨ BC required a blender but easy to implement
- ⇨ BC is more reproducible

Experimental comparison : (Pros/cons)

- ⇨ Samples homogeneity similar for sample analysed in single and in totallity
- ⇨ BC most adapated for samples analyzed in duplicate
- ⇨ BC enable to assess the subsampling step in Interlaboratory Proficiency testing trial
- ⇨ BC is particularly appropriate to prepare enough samples needed for ILPT
- ⇨ Level contamination range for BC is suitabled to prepare the samples for ILPT, especially for the low detection level
- ⇨ Samples homogeneity with BC remains the most satisfactory

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