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

Laboratory for Food Safety
Maisons-Alfort location

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

Improvement of initial contamination mode to conduct challenge test for *Listeria monocytogenes*

Anne-Laure Lardeux

9th workshop of the EURL for *Listeria monocytogenes,*

25-27 March 2015

French Agency for Food, Environmental and Occupational Health Safety – Food Safety Laboratory
Maisons-Alfort location and Boulogne-sur-Mer location
14, rue Pierre et Marie Curie - F94701 Maisons-Alfort Cedex - Telephone: + 33 149 771 350 - Fax: + 33 149 771 380
Boulevard du Bassin Napoléon - F62200 Boulogne-sur-Mer - Téléphone : +33 321 992 500 – Télécopie : + 33 321 991 725
www.anses.fr - @Anses_fr

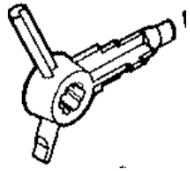
Context

- NRLs WG (2012-2014: revision of the EURL *Lm* Technical Guidance Document for conducting shelf-life studies)
  →identification of the need for investigating and recommending new techniques for the initial contamination of food samples. One of the most important aspects = to improve the simulation of the real (natural) contamination.
- In the current version of EURL Lm TGD: some ex. of initial contamination techniques but not the contamination with an airbrush (more largely used), by lack of data concerning the precision of this method at the moment of diffusion.
- → Main goal = to collect data to assess the performance of the airbrush technique.
- → Study of initial contamination with an airbrush in comparison with a contamination by spots on agar surface.

Description of the airbrush kit, model A430 (Aztek)

Airbrush

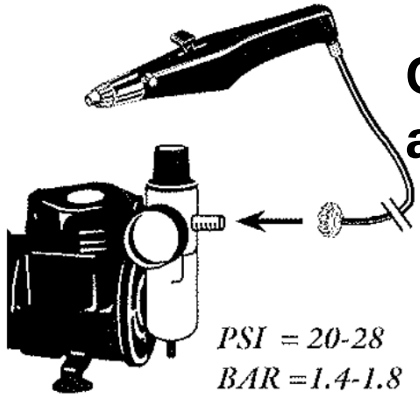
Nozzles (a gray: 40mm, a turquoise: 50mm and a red: 53mm)



Nozzle wrench

Gravity feed cup

Siphon caps



Compressor adapter

Air compressor

Determination of flow

- For spraying liquid from airbrush, it is necessary to use the trigger.

- In order to have a fixed flow, it is necessary to have a fixed stop.
  Initial stop → spraying a too big quantity of liquid in a too fast time.
  ⇨ a new stop to obtain an appropriate flow.
  ~~red nozzle~~.
- Tightening of the nozzle = impact on the flow → tighten it at the max and then to loosen the equivalent of a notch (= standardise the flow).
- Measurement of the flow of the 2 other nozzles:

Nozzles	Flow (ml/s)	Flow (seconds for 1 ml)
Gray nozzle	0.017±0.002	59.3±6.6
Turquoise nozzle	0.043±0.004	23.7±2.3

Preliminary tests

- Before using bacteria to test the airbrush, use of a dye, methylene blue, to study:
  - the nozzles,
  - the appropriate pulverisation distance,
  - the choice of the more appropriate cap.

Tested parameters	Observation	Conclusion
Pulverisation speed according to the used nozzle	Turquoise nozzle: a too fast liquid pulverisation	The gray nozzle
Pulverisation distance	10 cm : an unappropriated pulverisation distance → an observed lack of pulverisation precision	A 5 cm pulverisation distance
Choice of the more appropriate cap	~~Siphon caps~~ → pulverisation of the partial quantity of liquid	Gravity feed cup → the pulverisation of the whole quantity of liquid

Preliminary controls

- Use of a non-pathogenic strain, of *Lactobacillus sakei*.
- **CONTROL OF AIRBORNE CONTAMINATION :** under a µbiological safety cabinet. Use of an air sampler for µbiological monitoring to check the absence of pulverisation of bacteria by the airbrush in the atmosphere of the safety cabinet. No bacterion grew on non-selective agar.
- **CONTROL OF AIRBRUSH DISINFECTION**:

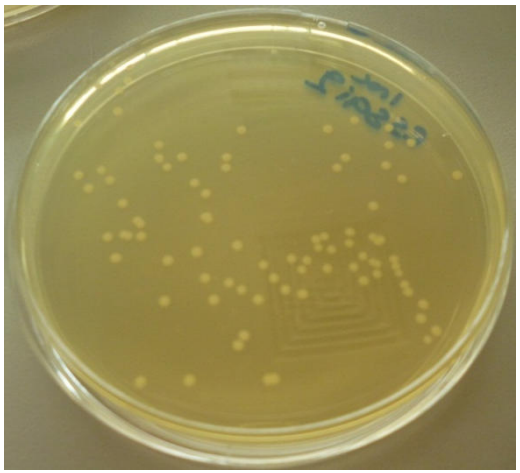
Control period	Methods	Results
Before each use	Dipping of the head of the airbrush (the slide on contact with the nozzle), gravity feed cup and nozzle during few seconds into successive bathes: alcohol 70° then distilled water and finally diluent	
Before the 1st pulverisation	Pulverisation of 200 µl of diluent through the assembled airbrush on a non-selective agar	No colony
After each use	Dipping of the head of the airbrush (the slide on contact with the nozzle), gravity feed cup and nozzle during different times into successive bathes: disinfectant (Surfanios or Amphospray) during 3 different tested times (15, 20 and 30 min), then during few seconds for alcohol 70° and distilled water.	1 colony after 15 min, 2 colonies after 20 min, no colony after 30 min in disinfectant bath. Conclusion: an at least 30 minutes dipping period for disinfectant bath

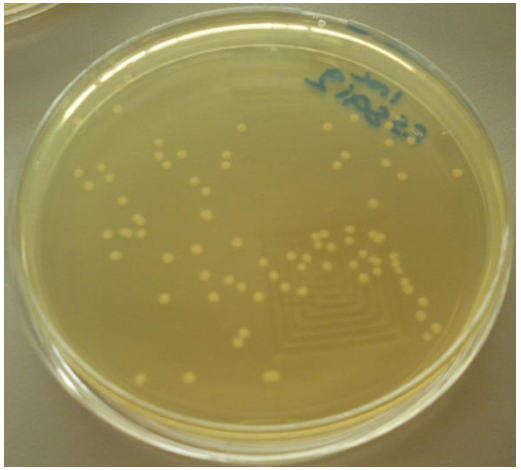
Comparison with classical spreading (1/2)

- Pulverisation of the bacterial suspension compared to a classical spreading, with a spreader at the agar surface.
- On 90-mm Petri dishes containing TSAYe, 300 µl of the appropriately diluted bacterial suspension distributed for each method.

Methods	Results of the 1st reproduction	Results of the 2nd reproduction
Classical spreading	52 and 56 cfu	84 and 90 cfu
Pulverisation spreading	56 and 100 cfu	72 and 87 cfu
Conclusion	Equivalent enumeration and dispersion on TSAYe surface	

Classical spreading





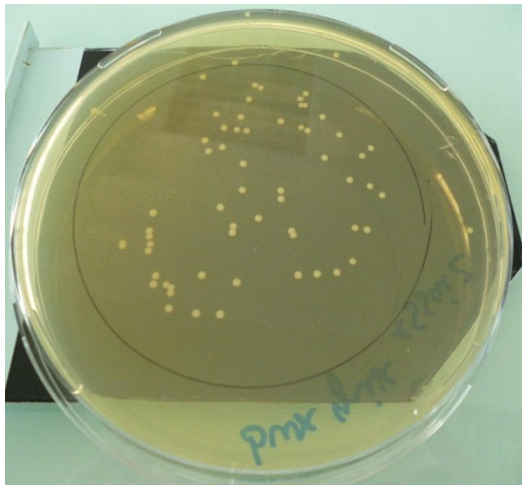
Pulverisation spreading

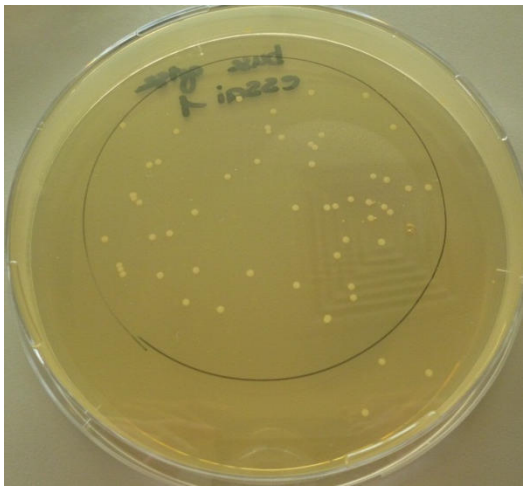
Comparison with classical spreading (2/2)

- Experiments performed with 140-mm Petri dishes containing TSAYe and a targeted area corresponding to a 90-mm diameter circle.
- Aim = to know if the pulverisation is well-oriented.

Method	Number of colonies in the targeted area	Number of colonies outside the targeted area
Pulverisation spreading	80, 59, 53 and 73 cfu	4, 7, 6 and 3 cfu
Conclusion	Percentage of colonies outside the targeted area = from 4% to 11%.	

4 colonies out the targeted area





3 colonies out the targeted area

Adequate pulverisation volume (1/2)

- Interesting to be able to pulverise small volumes of bacterial suspension, while keeping the targeted value of initial concentration, without modification of the a_w of the tested matrix (max 1% between V_{inoc} and the M or V_{matrix}).
- On 90-mm Petri dishes, a reference bacteria concentration with a 100 µl classical spreading (with a spreader at the agar surface) = $8.7.10^8$ cfu/ml.
- Pulverisation technique tested to compare with the former technique:

	Pulverisation volumes spread on 90-mm Petri dishes		
	300 µl	200 µl	100 µl
Concentration obtained by pulverisation spreading	1.10^9 cfu/ml	$1.2.10^9$ and $9.8.10^8$ cfu/ml	5.6 and $7.2.10^8$ cfu/ml
Conclusion	Satisfactory precision. 100 µl: too fast pulverisation in order to allow a good repartition on the contaminated surface. 200 µl: retained because of a not too fast pulverisation and a not too important adsorption volume.		

Adequate pulverisation volume (2/2)

- Reference bacteria concentrations obtained by classical spreading (100 µl of the suspension at the agar surface on 90-mm Petri dish) = respectively $6.2.10^8$ cfu/ml and $6.8.10^8$ cfu/ml.
- On large agar plates (38x28 cm), pulverisation technique tested to compare with the former technique:

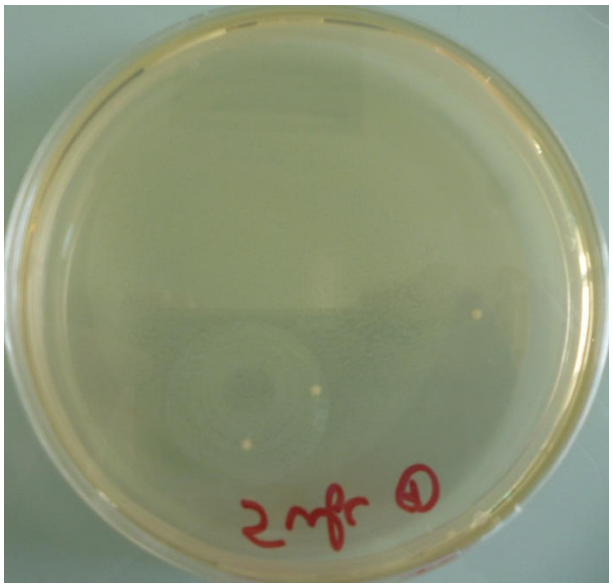
	Pulverisation volumes spread on large agars (38x28cm)		
	1 ml	2 ml	6 ml (maximum volume of the studied gravity feed cup)
Concentration obtained by pulverisation spreading	$4.8.10^8$ cfu/ml	$5.0.10^8$ cfu/ml	
Conclusion	Correct concentration in comparison to the one obtained by classical spreading	Correct concentration in comparison to the one obtained by classical spreading.	Volume not appropriate to respect the EURL Lm technical guidance document
	No difference between results obtained with the volumes 1 ml and 2 ml, so equal use of these 2 volumes		

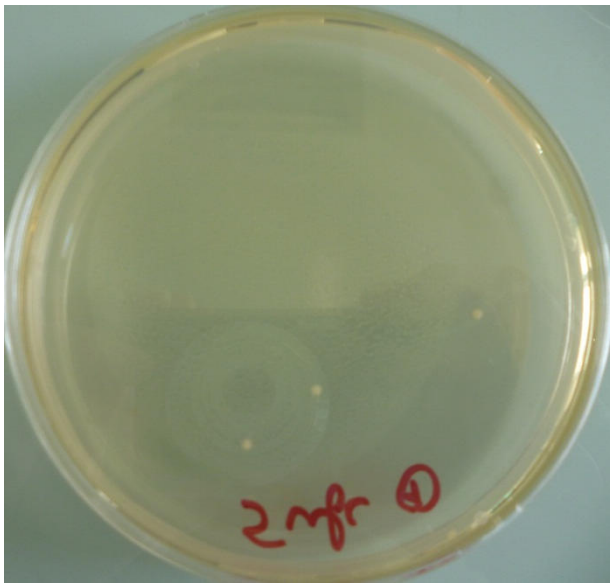
Possibility to pulverise a precise small quantity of bacteria

- One of the aims of the study
- Pulverised volume = 200 µl. Aim = reach 5 or 15 cfu on the surface of a 90-mm Petri dish.

Targets	5 cfu	15 cfu
Results	7, 1, 4, 6, 2 and 3 cfu	11, 12, 10, 14, 9 and 15 cfu
Conclusion	Possible use of pulverisation technique to contaminate a matrix at a precise small bacterial concentration	

4 cfu vs 5 cfu targeted





12 cfu vs 15 cfu targeted

Comparison between 2 matrix contamination techniques

- Technique currently used at the lab to contaminate a slice of smoked salmon = take 100 µl of the inoculum at the appropriate dilution, then to deposit 5 spots of 20 µl on ½ of the slice, to fold over the other ½ of the slice and to use a spreader to improve spreading of the inoc on the matrix.
- Comparison with the pulverisation technique on TSAYe.

Methods	Deposit of 5 spots of 20 µl on a targeted area without spreading	Deposit of 5 spots of 20 µl on a targeted area with spreading
Classical spreading	$6.4.10^8$ cfu/ml	$5.5.10^8$ cfu/ml
Pulverisation of 100 µl on a targeted area	$5.6.10^8$ cfu/ml	$5.3.10^8$ cfu/ml
Conclusion	Slightly larger number of colonies with the classical spreading Hypothesis: less loss of the colonies on material surfaces with the classical spreading because of a unique contact with the tip of the pipette vs contact with the tip of the pipette, the gravity feed cup and the airbrush with the pulverisation spreading	An equivalent number of colonies with both techniques and a correct repartition of these colonies on the limited area. Drawback of the classical technique with spreading: spreading not always possible, according to the studied matrix.

Conclusion

- Contamination technique by airbrush pulverisation = can be used for artificial contamination in the frame of challenge tests as well as PT trials, for *Lm* as for other bacteria.
- 2015: technique implemented by EURL CPS to contaminate solid food matrices (cheeses) in the frame of the organisation of future PT trials so as to optimise the artificial surface contamination, allowing to include a sub-sampling step of the test portion (step with a major impact on the validity of the analyses of solid matrices).
- Technique: to help EURL and NRLs to implement easily an artificial contamination technique, while improving the repeatability and ensuring a satisfactory homogeneity and stability of bacterial contamination.

Thank you for your attention