



WETENSCHAPPELIJK INSTITUUT
VOLKSGEZONDHEID
INSTITUT SCIENTIFIQUE
DE SANTÉ PUBLIQUE



Practical expertise in proficiency testing trials at NRL Belgium

Workshop *L. monocytogenes* - March 2015

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Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

Article 33

National reference laboratories

1. Member States shall arrange for the designation of one or more national reference laboratories for each Community reference laboratory referred to in Article 32. A Member State may designate a laboratory situated in another Member State or European Free Trade Association (EFTA) Member and a single laboratory may be the national reference laboratory for more than one Member State.
2. These national reference laboratories shall:
 - (a) collaborate with the Community reference laboratory in their area of competence;
 - (b) coordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples in accordance with Article 11;
 - (c) where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing;

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Mainly dedicated to laboratories approved by the Federal Agency for the Safety of the Food Chain (FASFC – Competent Authority): around 20 labs.



Features:

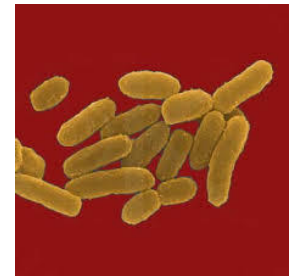
- fresh matrices (naturally contaminated by **background flora**)
- samples contaminated with a mixture of bacteria

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3 PT/year:

- March: *Y. enterocolitica* and pathogenic *E. coli* **detection** on carcass swabs.

2015: sprouts.



- November: *Campylobacter* spp **enumeration** in chicken products

+ *E. coli* ESBL
inoculation



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- June: *L. monocytogenes*, *B. cereus*, *E. coli*, *Pseudomonas* spp, coagulase positive *Staphylococcus* (CPS) **enumeration** in a different matrix each year (pâté, beans, smoked salmon, pastry cream and fresh cheese).

2015: surimi



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Contamination of the samples

Performed at our laboratory

2 different techniques tested:

- 1° → lyophilized culture : added to the sample by the participant just before starting the PT
- 2° → fresh culture: added in the laboratory 1 or 2 days before starting PT, depending on the bacteria

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Contamination of the samples

Lyophilized culture

Advantages: long stability at fridge or room T°

Disadvantages: not a real sample, difficulty to reach exact levels of concentration (mainly low levels) → not optimal



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Contamination of the samples

Fresh culture

Contamination by the NRL either directly in the stomacher bag used by the participant to start the PT or in a big portion of matrix (later divided in small portions)



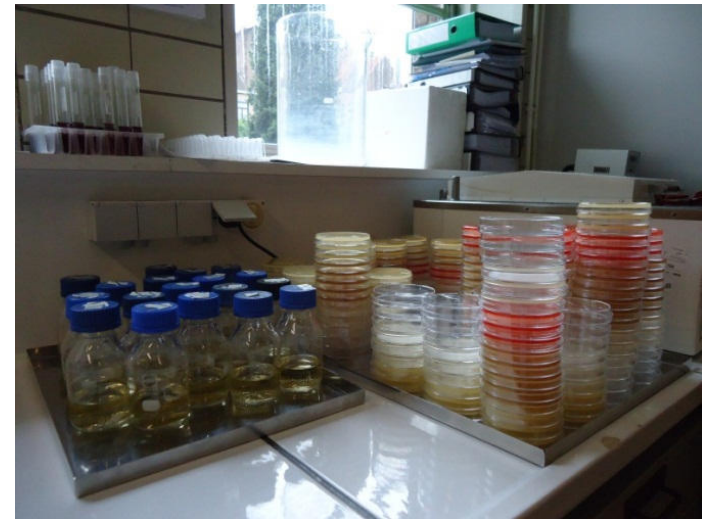
Advantages: real sample, possibility to inoculate at 3 levels for enumeration PT (blank, low and medium)

Disadvantages: not very long stable → participants have to start the analysis at D+1 or D+2 maximum (lack of flexibility)

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Laboratory work:

- some tests needed to reach the right concentrations (difficulty with combinations of bacteria)
- homogeneity tests (day D0)
- stability tests (D start PT)



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Combination bacteria-matrix

- Homogeneity tests: always OK
- Stability tests: depending on the matrix. Sometimes difficulties oa. *B. cereus* and CPS in fresh cheese.



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In the transport box:

- Stomacher bags or small containers
 - precise weight of the sample to start the analysis
 - higher weight (→ sub-sampling necessary)
- Datalogger
- Ice-block

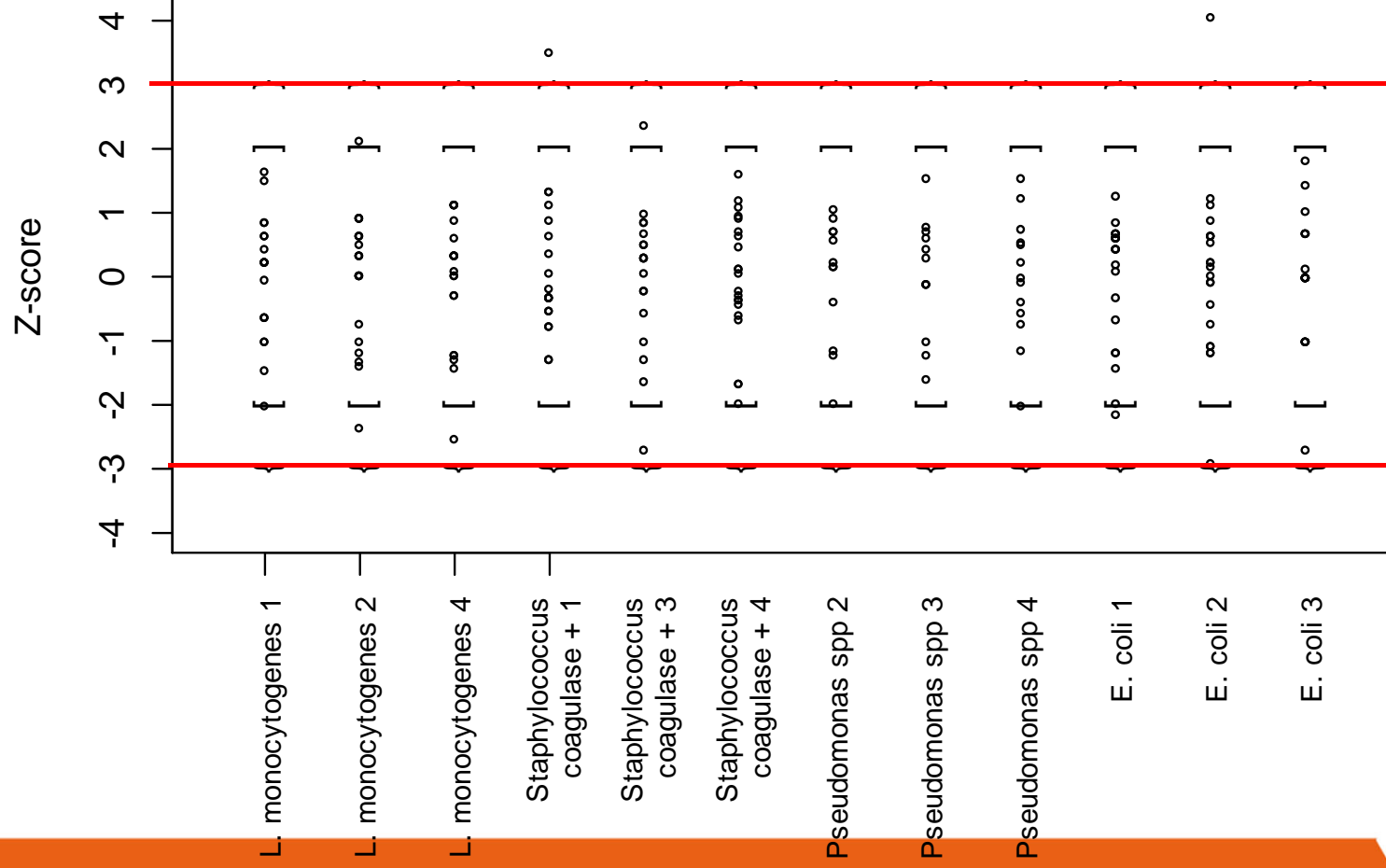


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Statistics

- Performed by the Service “Quality of medical laboratories” at WIV-ISP
- Performance of the labs: calculation of **z-score** according to the robust average and robust SD of the participant’s results (ISO 13528)
- No repeatability calculation

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PT report and follow-up of the labs

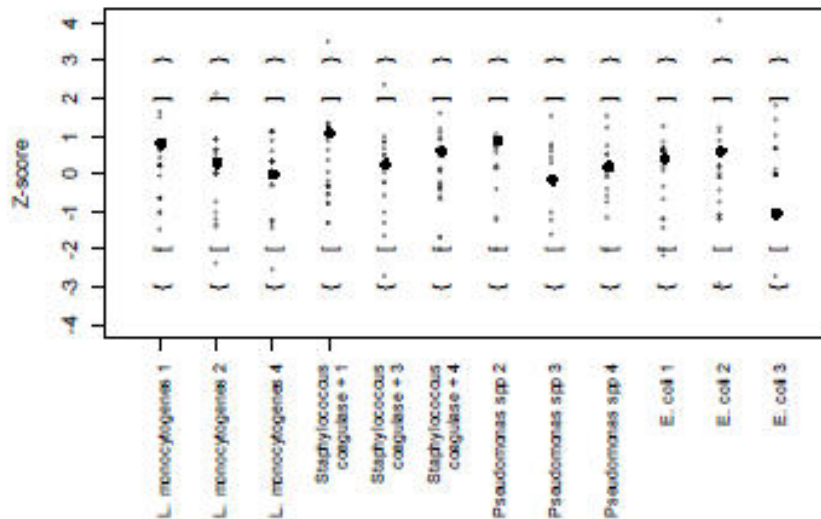
- Intermediate report: within 1 month, z-score
- Final report: within 3-4 months
- Follow-up of the labs with unsatisfactory z-score
- Evaluation of the PT organisation by the labs



Results proficiency test enumeration 2014

Your results

Sample	Your result	Log10-value	Robust mean	Robust standard deviation	Z-score
L. monocytogenes 1	130	2.114	1.967	0.18	0.818
L. monocytogenes 2	1400	3.146	3.116	0.098	0.307
L. monocytogenes 4	1190	3.076	3.078	0.116	-0.022
Staphylococcus coagulase + 1	110	2.041	1.843	0.182	1.093
Staphylococcus coagulase + 3	70	1.845	1.772	0.283	0.259
Staphylococcus coagulase + 4	450	2.653	2.524	0.204	0.631
Pseudomonas spp 2	800	2.903	2.615	0.328	0.877
Pseudomonas spp 3	300	2.477	2.508	0.222	-0.139
Pseudomonas spp 4	3100	3.491	3.428	0.294	0.214
E. coli 1	3800	3.58	3.523	0.134	0.42
E. coli 2	420	2.623	2.522	0.163	0.625
E. coli 3	20	1.301	1.485	0.178	-1.039



Black dots show your Z scores within the 3Z-limits, red dots show your Z scores outside the 3Z-limits. Small circles are the Z scores from the other laboratories

RAPPORT FINAL

ESSAI D'APTITUDE EN MICROBIOLOGIE ALIMENTAIRE
JUN 2014

MATRICE: FROMAGE FRAIS


DENOMBREMENT

Secteur: Pathogènes alimentaires
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New website

- Registration to an annual campaign **OK**
- Submission of results **OK**
- Access to the intermediate report **coming soon**
- Access to the final report **coming soon**
- Link PT-scheme (FAFSC) **OK** 

Started in November 2014

.be



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Essais d'aptitude microbiologie alimentaire (EAMA)

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Essais d'aptitude microbiologie alimentaire (EAMA)

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Titre	Période	Statut	Action
Détection de E. coli pathogène dans les graines germées	Mars 2015	Initialisé	Aucune action disponible
Dénombrement de E. coli, Staphylococcus Coagulase positive, L. monocytogenes, Pseudomonas spp et B. cereus dans la salade de surimi	Juin 2015	Initialisé	Aucune action disponible
Dénombrement de Campylobacter spp	Novembre 2015	Initialisé	Aucune action disponible

- Inscriptions
- Soumettre résultats

Détection de E. coli pathogène dans les graines germées

Données administratives +

Résultats -

	E. coli
Echantillon 1	DéTECTÉ/25g
Echantillon 2	DéTECTÉ/25g
Echantillon 3	DéTECTÉ/25g
Echantillon 4	DéTECTÉ/25g
Echantillon 5	DéTECTÉ/25g

Remarque :

Conclusion

- All steps of the PT (sample preparation, distribution, of the samples, statistics ...) performed by the NRL
→ difficulty to follow completely the ISO norms 17043 and 13528. Too expensive and too much work for our small team.
- Each year a challenge because new matrix for the enumeration PT.
- Analysis of results always very interesting
- Easier now with our new web application.



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Thank you for your attention!



Questions?

