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EURL *LM* PROFICIENCY TESTS ON TYPING

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INVESTIGATE, EVALUATE, PROTECT

History

- Characterisation: Molecular serotyping anf Typing: PFGE for cluster analysis

And then including WGS methods in 2018:

- PT 2018 to PT 2021 the three WGS approach: SNP, cgMLST and wgMLST

In 2022 : PT cgMLST

ECDC and EFSA decided to select the Pasteur cgMLST schema for *Lm*

for their respective systems: TESSy and EFSA One Health WGS System

Current organisation under 17043 accreditation

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1)Preparation of PT items:

- Pre_test analysis: Panel of 4 strains and 6 fastq files (with FTP server)
  - depth of 30X min,
  - difference between assembly and reference genome < 20%
  - Coverage > 80%
  - no contamination (interspecies and intraspecie)

2)Homogeneity

- in triplicate with CC determination by PCR method GenoListeria

3) Stability (DNA extraction , sequencing and cluster analysis)

Request to NRLs *Lm*

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- Report file :Serotypes, MLST CC and cluster determination
- Quality control report in PDF files of raw data (for example fastqc script or Fastp)
- File of raw reads in fastq format with a minimum coverage of 20X
- Assembly fasta files
- For the cluster determination: matrix distance (.csv and/or .tsv format) & phylogenetic tree (newick format .nwk)
- Contaminated strains or contaminated fastq to be mentioned in the report file but not included in the final panel and results (matrix distance, phylogenetic tree and profiles allelic).

Evaluation of the PT results

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- Report file with all the parameters (serotype, cc, cluster determination and contaminated strain)
- quality assessment from sequences
- Statistical analysis
- Matrix distance:
  - evaluated by a matrix distance comparison (Mantel test) in comparison with reference matrix distance.
    -> Satisfactory if the mantel coefficient ≥ 0.70
- and phylogenetic tree:
  - Phylogenetic tree evaluated by a cophenetic distance
    -> Satisfactory if cophenetic correlation coefficient ≥ 0.70
- determine the serotype, the clonal complex and clusters by cgMLST method (threshold imposed to 7 difference allelic)
- **Satisfactory if all results assessed comply with the expected results: distance matrix, phylogenetic tree, serotype, clonal complex, contaminated strain and cluster determination**

Table 15. Summary of compliance with instructions and performance evaluation for each laboratory

Code Lab	Instructions						Wetlab Part				Drylab Part					Deviations
	Test report	fastqc	fastq.gz	Fasta	Matrix distance	Phylogenetic tree	DNA extraction	Library preparation	Sequencing	Detection of fastq contamination	Mantel coefficient	Cophenetic coefficient	Cluster identification	Serotype determination	CC determination	
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
7	S	S	S	S	S	S	S	S	S	S	NE	S	S	S	S	The Lab 7 has detected the contaminated fastq but it was included in matrix distance
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	The Lab 8 has not provided the ILPT results
9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
10	S	S	S	S	S	S	S	S	S	S	S	S	U	S	S	Cluster identification unsatisfactory
11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
12	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
13	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
14	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
17	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	The Lab 17 has not respected to perform the ILPT results with the cgMLST approach
18	S	S	S	S	S	S	S	S	S	S	S	S	U	S	S	Cluster identification unsatisfactory
19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	

S: satisfactory, U: unsatisfactory, ND: not done, NE: not evaluated

The results of 16 laboratories were assessed. Three participants (19%) obtained unsatisfactory results:

- Two participants did not detect the expected cluster (Lab 10 and Lab18).
- One Lab took into account the contaminated fastq in the matrix distance.

The results of 13 out of 16 evaluated laboratories were assessed satisfactory.

Upcoming

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- As reference pipeline established by EFSA
- Evaluation results lAssessment of the allelic profile (EFSA format) for individual strain identification
- Allowing limited number of strains to be sequenced