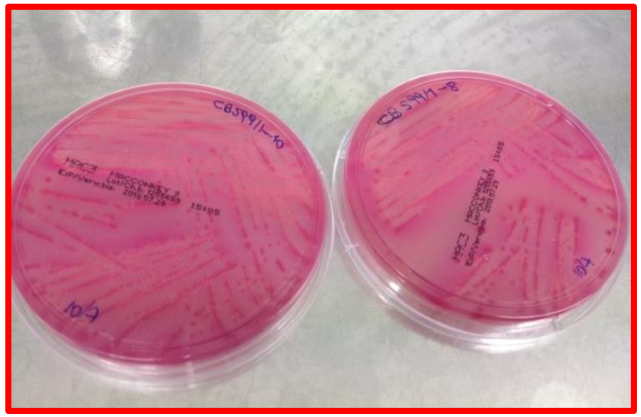
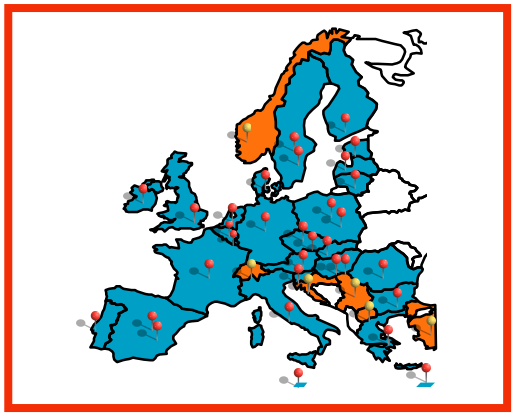
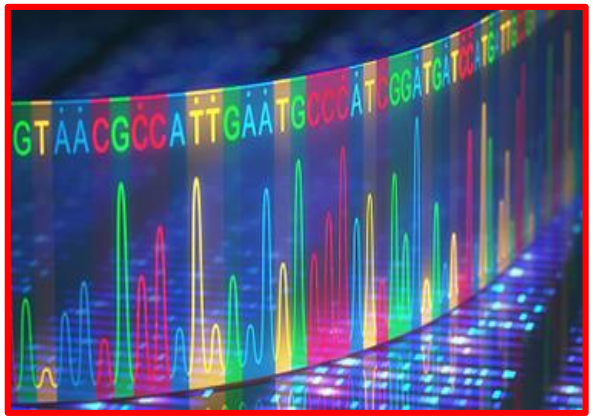
PT28

Identification and typing of Shiga toxin-producing *E. coli* (STEC)







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The objectives of the study were:

- The detection of the main STEC virulence genes (*eae* and *stx* genes)
- The identification of a range of relevant STEC serogroups
- The subtyping of Shiga Toxins (Stx)-coding genes
- The identification of clusters of isolates based on genomic analysis

PT28: Design of the study

1. Identification of the shiga toxin-producing *E. coli* main virulence genes by PCR amplification:

   ***stx1* type**, ***stx2* type** and the **intimin-coding *eae* gene**

2. Identification of **13 target O serogroups**:

   O26, O103, O111, O145, O157 ("top 5")
   O45 and **O121** (considered as adulterants in beef in the USA)
   O104 (relevant after the 2011 outbreak)
   O55, **O91**, **O113**, **O128**, **O146** (prevalent in human infections in Europe according to the ECDC data)

3. Subtyping of *stx* genes:

   stx1a, ***stx1c*** and ***stx1d***
   from ***stx2a*** to ***stx2g***

4. Comparison of genomic signatures

   determine the relatedness using **cgMLST** or **SNPs-based** methods

Main characteristics of the eight strains

Strain	Serotype	ST	Target virulence genes		
			stx1	stx2	eae
1	O80:H2	301	-	*stx2f*	+
2	O80:H2	301	-	*stx2a*	+
3	O80:H2	301	-	*stx2a*	+
4	O80:H2	301	-	*stx2a*	+
5	O80:H2	301	-	*stx2a*	+
6	O26:H11	21	*stx1a*	-	+
7	O146:H21	442	*stx1c*	*stx2b*	-
8	O104:H7	2283	*stx1c*	-	-

Complete WGS-based virulotyping

Strain	Virulence Genes
1	*cea, cma, cvaC, eae, efa1, ehxA, espA, espB, espF, espP, gad, hlyF, hra, ironN, iss, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir*
2	*cma , cvaC, eae, efa1, ehxA espA, espB, espP, gad, hlyF, iha, ironN, iss, mchB, mchC, mchF, nleA, nleB, nleC, ompT, stx2, sitA, tir*
3	*cvaC, eae, efa1, ehxa, espA, espB, espF, espP, etsC, gad, hlyF, iha, ironN, iss, mchB, mchC, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir*
4	*eae, efa1, ehxA, espA, espB, espF, espP, gad, hlyF, hra, iha, ironN, iss, mcbA, mchB, mchC, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir*
5	*eae, efa1, ehxA, espA, espB, espF, espP, gad, hlyF, hra, iha, ironN, iss, mcbA, mchB, mchC, mchF, nleA, nleB, nleC, ompT, sitA, stx2, tir*
6	*astA, cia, cib, cif, eae, efa1, ehxA, espA, espB, espF, espJ, espP, gad, iha, iss, iucC, iutA, katP, lpfA, nleA, nleB, stx1, terC, tir, toxB, traT*
7	*cea, ehxA, epeA, espI, focC, gad, iha, ireA, ironN, iss, kpsE, lpfA, mcbA, mchB, mchC, mchF, mcmA, sfaD, stx1, stx2, subA, tia*
8	*aaiC, celB, epeA, gad, ireA, katP, lpfA, neuC, orf3, stx1*

PT28: Participants

28 NRLs representing
24 EU MS

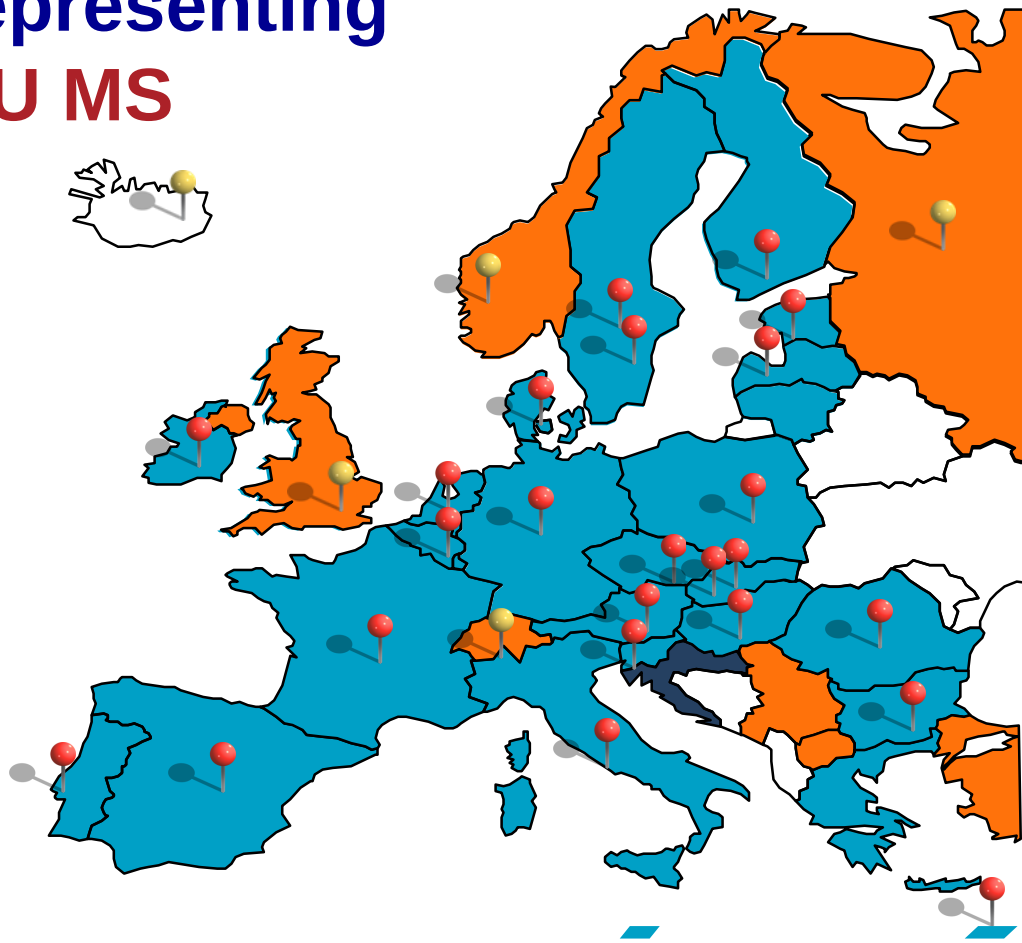
+ the NRLs of

Norway

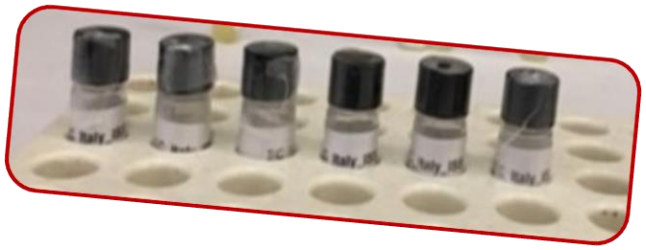
Russia

Switzerland

UK



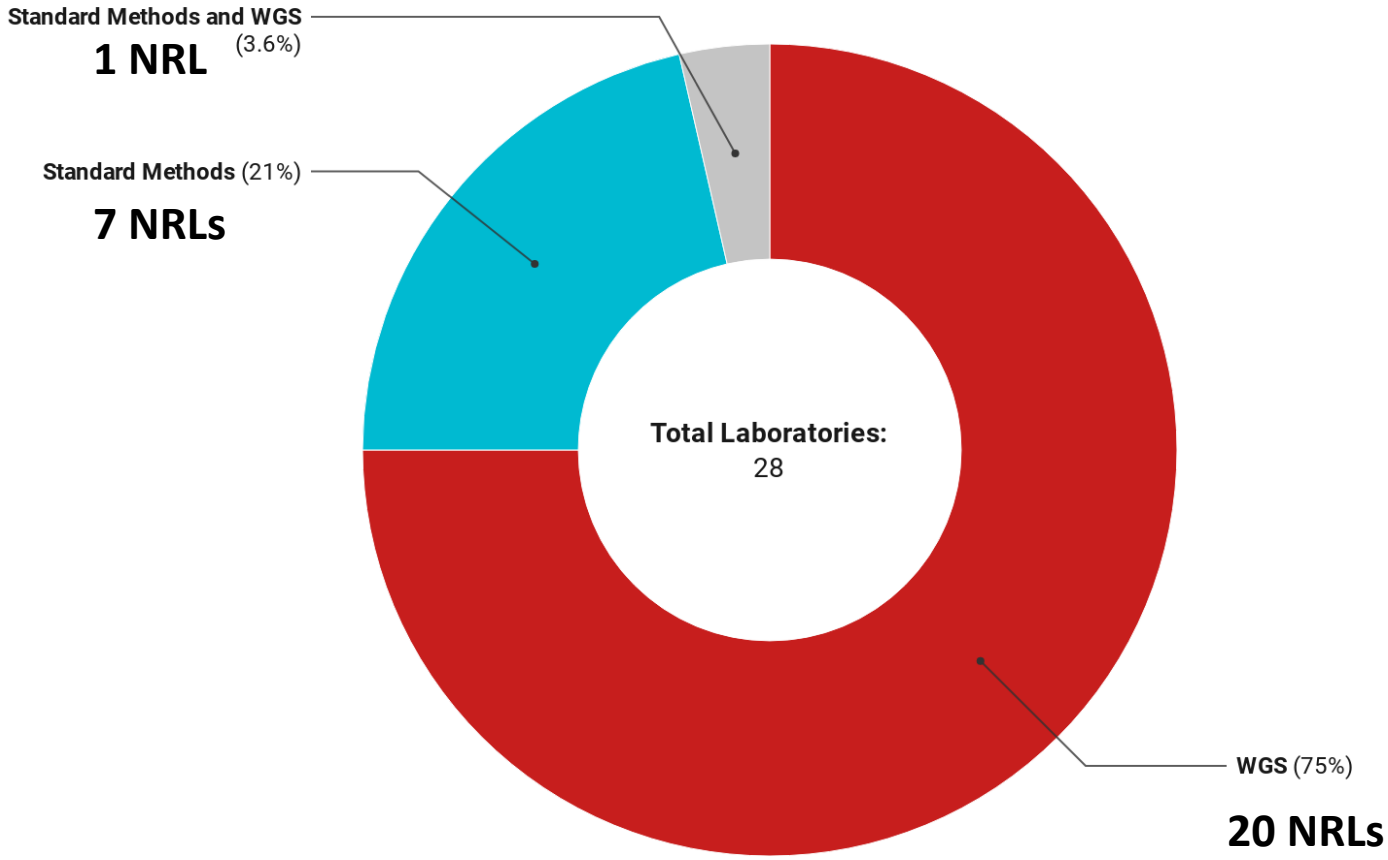
PT28: Samples



- ✓ 8 test strains as cultures in soft-agar
- ✓ Upon request, the needed control strains have been provided
- ✓ Test Samples were prepared on October 27th 2020
- ✓ October 28th 2020: the homogeneity test was performed on a set of 6 randomly selected samples
- ✓ Samples labelled with randomly generated numerical codes shipped on November 9th 2020
- ✓ Results submitted on-line via the web site by 28 NRLs

Number of laboratories reporting results/methods

Figure 1: Number of Laboratories reporting results obtained with the different analytical methods

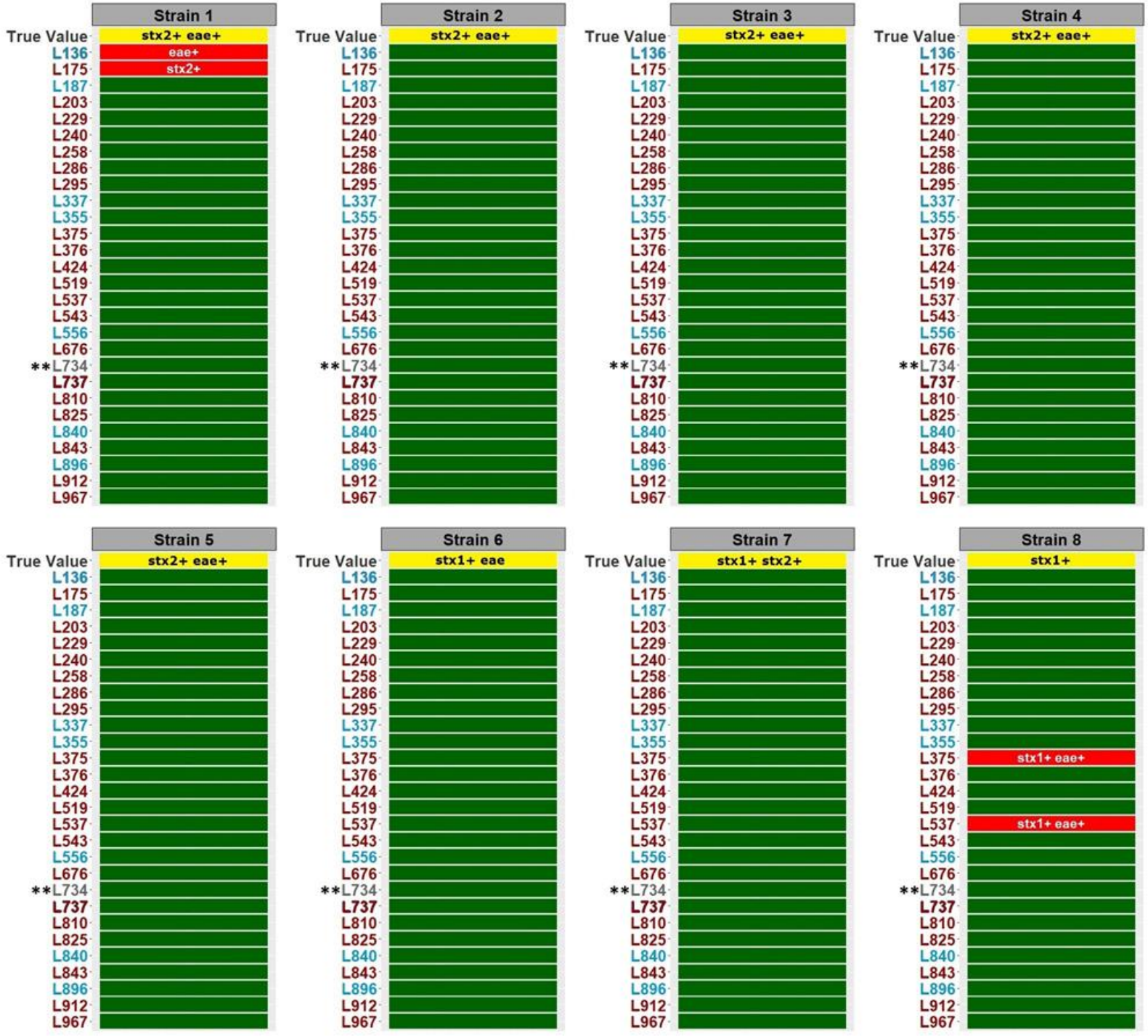


Penalty Points for the identification of STEC virulence genes and serogroups

- **4 penalty points** to each incorrect or missing result concerning the identification of the *stx* genes
- **2 penalty points** to each incorrect or missing result concerning the identification of *eae* gene
- **2 penalty points** to each incorrect result concerning the identification of serogroups
- **1 penalty point** when the results of the serogroup identification were not uploaded ("null" field) or reported as "Not Done"
- **1 penalty point** to each incorrect result concerning the identification of the *stx* genes subtypes (not considered for the assessment of the laboratories' proficiency)

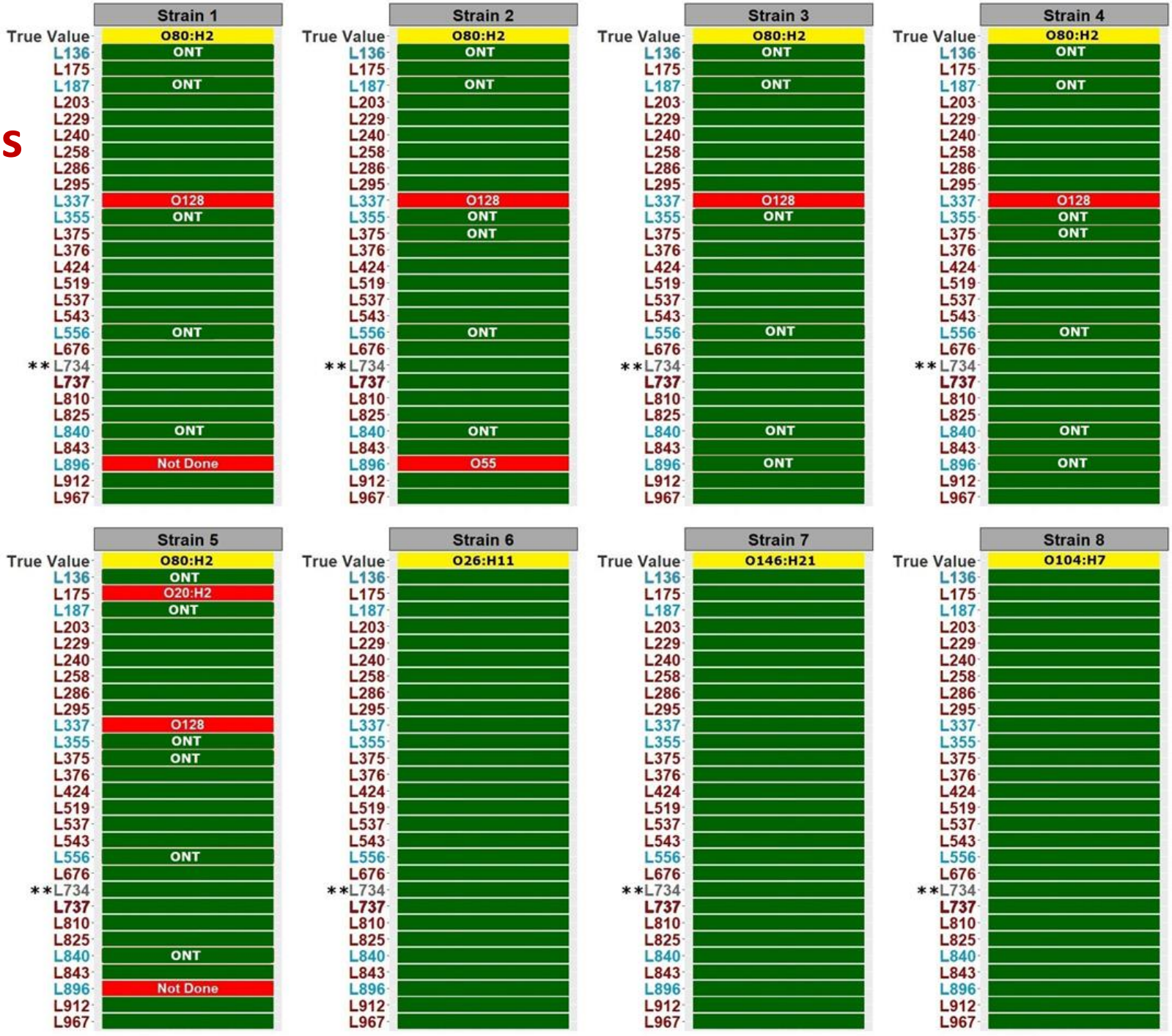
A threshold of 8 penalty points was set in order to identify the under-performant laboratories

Results virulence genes



Results

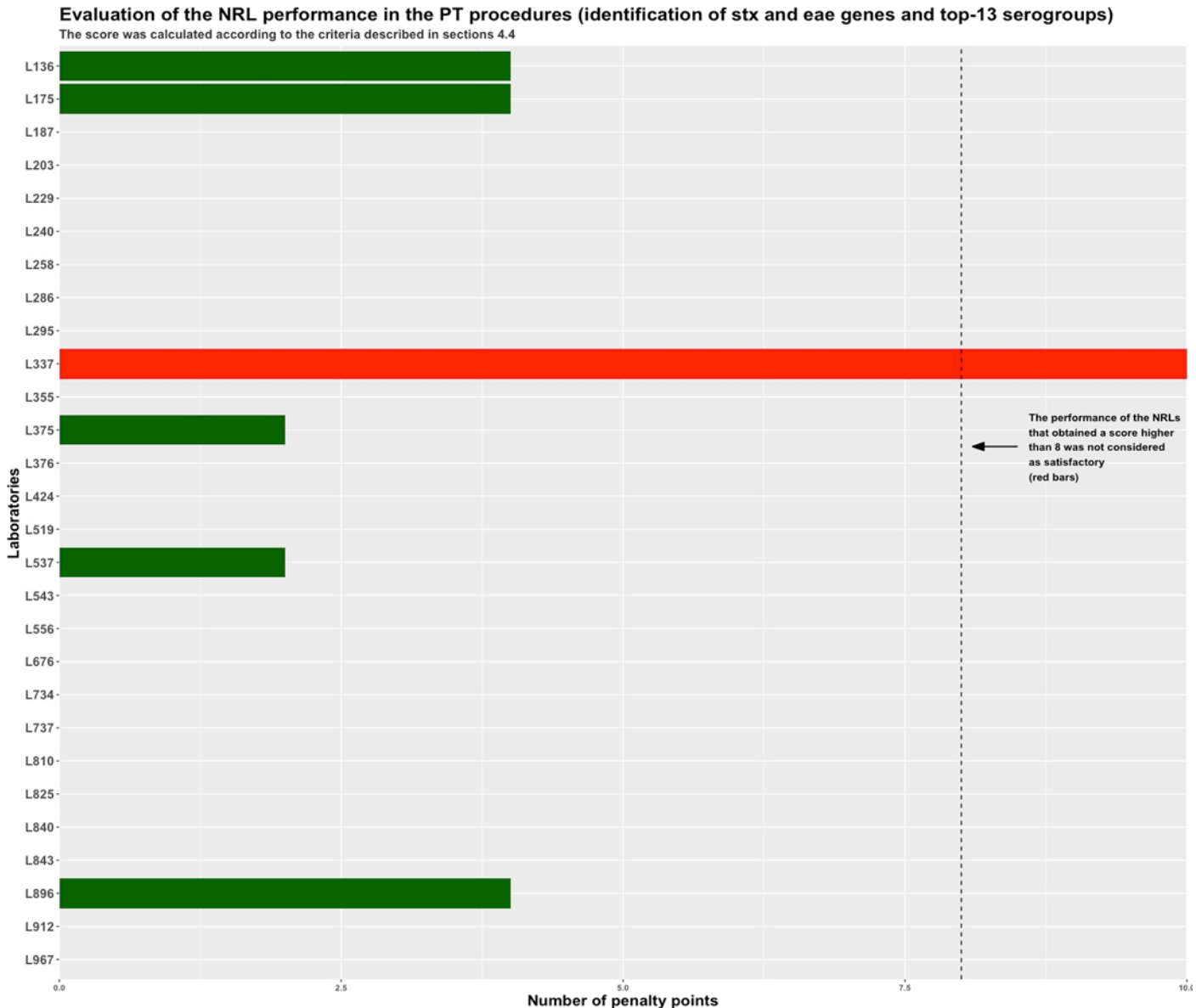
serogroups



Evaluation of the laboratories' performance

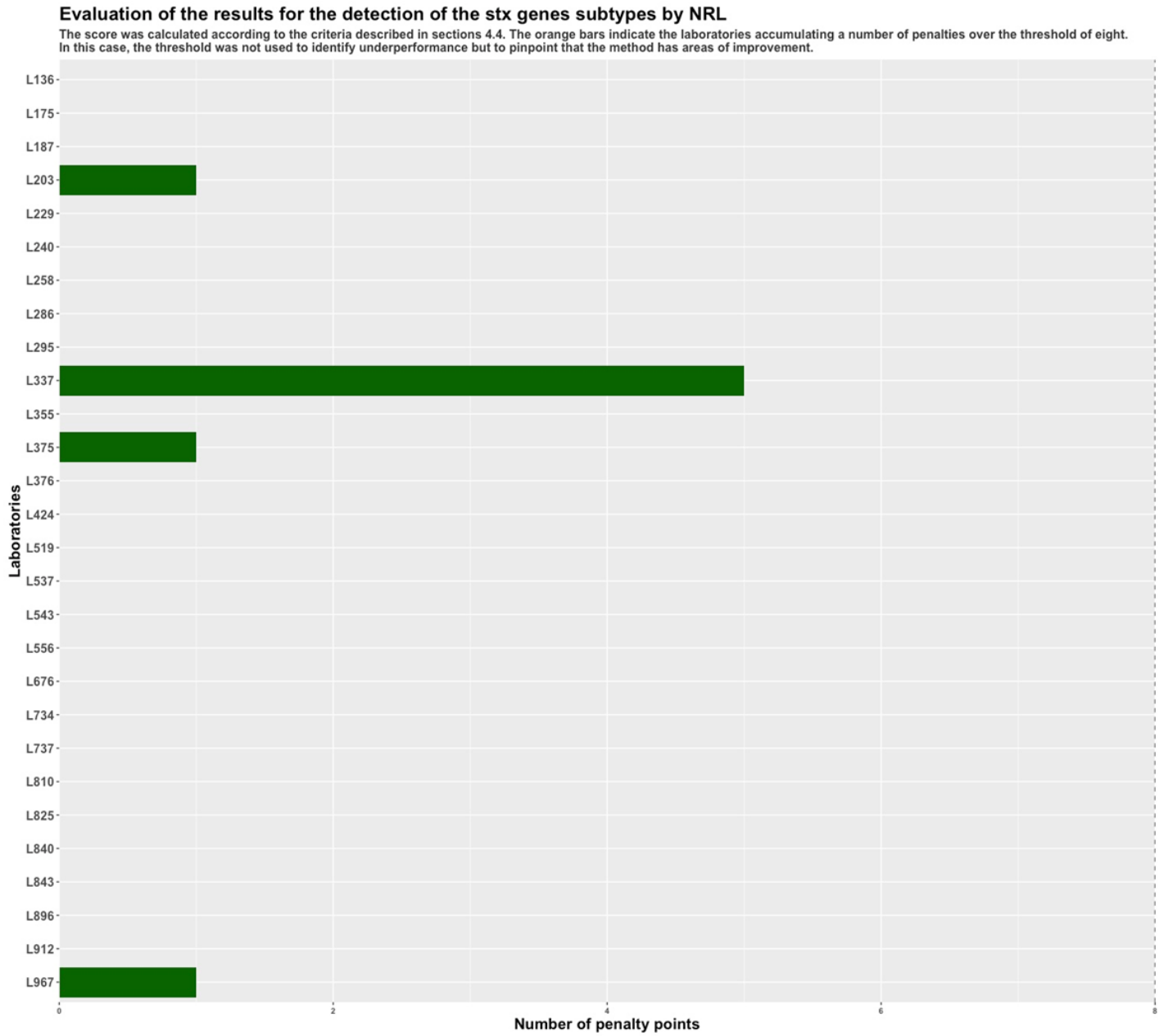
Identification of *stx, eae* genes and serogroups serogroups

The red bar indicate the NRL whose performance was considered as not satisfactory



Results *stx* subtyping

Evaluation of results of *stx* genes subtyping



Cluster Analysis

Voluntary exercise: performing cluster analysis on the 8 test strains

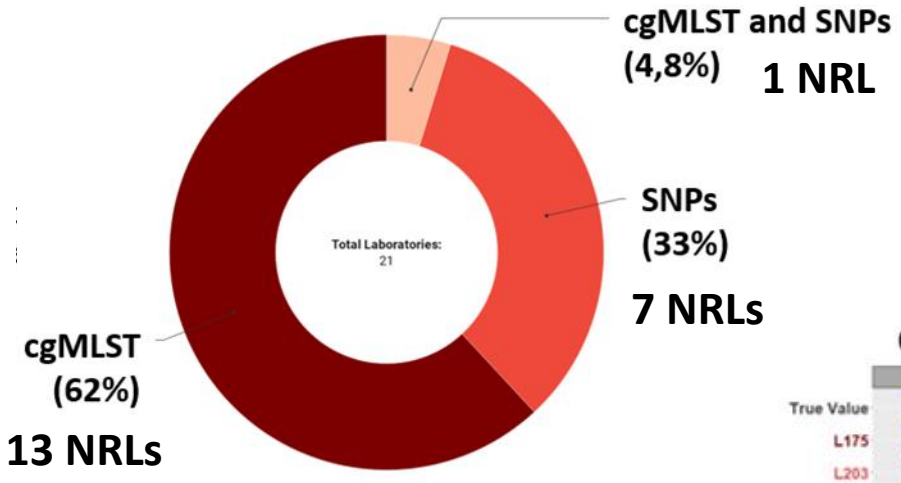
Methods: Only WGS-based: SNPs analysis or cgMLST (or both)

Submit info on the strains forming a cluster (strains IDs and maximum allelic or SNPs differences in the cluster)

No .fastq, .fasta files, trees or distance matrices submitted

Interpretation of the cluster performed by the NRLs

21 NRLs partecipated in this exercise



Strains 1, 2 and 3 all showed more than 45 allelic differences in cgMLST

CLUSTER ANALYSIS RESULTS

True Value	CLUSTERING STRAIN 4 - 5 with Allelic Differences = 0
L175	
L203	
L229	
L240	
L258	CLUSTERING STRAIN 1 - 3 - 4 - 5
L286	
L295	CLUSTERING STRAIN 1 - 2 - 3 - 4 - 5
L375	CLUSTERING STRAIN 1 - 2 - 3 - 4 - 5
L376	
L424	
L519	
L537	
L543	
L676	
L734	
** L737	
L810	CLUSTERING STRAIN 1 - 2 - 3 - 4 - 5
L825	
L843	
L912	
L967	

DISTANCE

Allelic Differences	SNPs
2	
	0-5
	0-15
0	
0-15	
3	
0-50	
	0-97
	0-1
2	
0	
0-5	
0	
0	
0	
0	3
	0-1451
0-5	
	0
0-7	
0-3	

PT28: Concluding Remarks

- ✓ **Lower participation compared to previous rounds, still good in pandemics time**! More than 75% performed WGS, displaying excellent performance in both the characterization and subtyping of the STEC isolates
- ✓ One laboratory underperformed in the characterization of STEC strains (detection of *stx*, *eae* and serogroups). **More than 95% of NRLs with correct results!** Identification of areas of improvement
- ✓ **Good identification of serogroups not covered by ISO TS 13136:2012**, especially when WGS was used
- ✓ **Good performance** on average for the ***stx* subtyping**. Apart from known criticalities of the typing method (e.g. discrimination between *stx2a* and *stx2c* genes in the PCR assay) the network responded well to the stx subtyping exercise, **particularly through WGS**
- ✓ 75% of NRLs performed **cluster analysis**, all those using WGS. **Good performance. Need for a threshold**, especially for SNPs analysis